Campden BRI
food and drink innovation

FINAL REPORT

Systematic and critical review on the potential use of bacteriophage on foods

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EXECUTIVE SUMMARY

The objectives of this review were to: establish the global use of bacteriophage; and carry out a critical review of the potential use of bacteriophage on foods, to act as an additional control for food pathogens and spoilage organisms within the supply chain.

An initial literature review revealed 6 key findings on the use of bacteriophage in the food industry to date:

Key finding 1: Generally Regarded As Safe (GRAS) approved, commercially produced bacteriophage are being used as food additives in the US and as processing aids in parts of Europe. The products are being used to reduce the pathogen load of *L. monocytogenes*, *Salmonella enterica* and *E. coli* O157:H7 on a range of named food types; poultry, red meat, RTE foods, fish, shellfish as well as processed fruits and vegetables.

Key finding 2: The major use of commercially produced bacteriophage to date is in the pre-harvest control of foodborne pathogens including STEC and *Salmonella*.

Key finding 3: Although bacteriophage are being approved for use in the food industry, one of the main barriers to their wider use is the formulation of guidelines for their safe and effective use. The three key safety concerns are: toxicological safety; emergence of resistance to biocides and antimicrobials linked to the use of bacteriophage; and the risk of release of bacteriophage into the environment.

Key finding 4: Research on the use of bacteriophage in the clinical sector is available, however official guidelines/regulations are yet to be published for bacteriophage therapeutic products in the US or Europe.

Key finding 5: A peer reviewed expert consensus on the quality and safety requirements is available for therapeutic products which could be modified for use in the food industry.

Key finding 6: At present there are no specific controls in place that are specific to the use of bacteriophage treatments on foods. There are however several regulations that must be complied with when using bacteriophage treatments in/on food products across Europe:

i) Hygiene regulation (EC) No 853/2004 applicable to bacteriophage treatments that are to be applied to the surface of meat or fish to remove bacterial contamination;
ii) Food Additive Regulation (EC) No 1333/2008 detailing controls specific to food additives and food processing aids; and
iii) General food law which states that food must be safe for human consumption

A critical review was carried out to collate data on the current research on the application of bacteriophage as biocontrol agents on foods. Information was gathered for: 5 key food pathogens (*Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, pathogenic *Escherichia coli* and *Staphylococcus aureus*); and 5 key groups of spoilage organisms (Gram negative rods, the Enterics, Gram positive spore formers, Gram positive non-spore formers and lactic acid bacteria). Following data analysis, scientific conclusions were drawn from the data to address the following points.

- Food products compatible with bacteriophage treatments
- The efficacy and specificity of bacteriophage
- The potential for the use of bacteriophage to protect against recontamination
- The safety of bacteriophage on foods and in food production
- Limitations on use of bacteriophage
- Any potential risks or issues with their use such as the possible emergence of bacterial resistance to bacteriophage treatments
- Whether bacteriophage is effective at point of application only (used as a processing aid), or whether it remains active over shelf life (i.e. considered to be an additive).

Analysis of the data revealed that bacteriophage treatments are used across the world to control foodborne pathogens, with the predominant use being pre-harvest applications. To date, there are no published guidelines on the use of bacteriophage in the food industry, however many of the practices used in the production of bacteriophage for therapeutics could be modified.

Research on the ability of bacteriophage to reduce pathogen load on foods has been published for the key foodborne pathogens, specially *Listeria monocytogenes*, *Salmonella* spp. and shiga toxin producing *E. coli* (STEC). Some studies have been published on the control of *Staphylococcus aureus* in dairy products using bacteriophage treatments. Although a significant number of papers are available on the control of *Campylobacter* spp. with bacteriophage, this work has focussed on live animals and in biofilms which were outside the scope of the study. Limited information is also available on spoilage organisms, however the majority of the literature assessed duing the review failed to reach the set threshold for inclusion in the evidence tables. Analysis of the data indicated that there is a potential to use bacteriophage to control levels of food borne pathogens, although additional work is required to establish the suitability of bacteriophage treatments for spoilage organisms.

One of the key points of focus of the review was the efficacy of the bacteriophage, which is governed by several factors: specificity of the bacteriophage; the potential for bacteriophage resistant mutants to be generated; and the capability of the bacteriophage to protect the food product over life. The capability of bacteriophage treatment to deliver protection against product recontamination is an important factor in deciding its function within the food product. There is insufficient data at present to support the use of bacteriophage to control the microbial load over product life and further work is needed to confirm if bacteriophage provide a one off 'kill' or are able to inactivate contaminating organisms introduced after the treatment period.

In addition to bacteriophage efficacy, safety considerations were highlighted on the use of bacteriophages as biocontrol agents on foods. The three key safety concerns were; toxicological safety; emergence of resistance to biocide and antimicrobials linked to the use of bacteriophage; and the risk of bacteriophage release into the environment. Of some concern in the literature was the impact of bacteriophage on toxin-producing bacteria and on how they may influence the human gut microflora. There are also concerns relating to the potential for the acquisition/transfer of virulence factors and/or genes from bacteriophage to either their target or additional hosts. It was proposed that knowledge acquired in the medical sector could be used to advise the development of guidelines and controls on the use of bacteriophage as biocontrol agents in foods.

Although there are many key benefits on the use of bacteriophages as biocontrol agents in foods, there are some limitations that should be taken into account. The food matrix plays a significant role in the success of bacteriophage treatments, and optimisation of the treatment is recommended to obtain maximum benefit. Amongst the parameters to take into consideration are the contact time and

temperature used for the bacteriophage treatment. Evidence also indicates that co-application of bacteriophage with another antimicrobial treatment often delivers an enhanced 'kill' of the target organism.

A list of conclusions drawn from the key findings highlighted in the report is listed below:

Conclusion 1: Although commercially available bacteriophage products are used to control microbial levels in the global food chain, further work is needed to establish and agree common guidelines for their safe and effective use. In some cases however bacteriophage treatments will require approval before they can be used in/on foods.

Conclusion 2: Knowledge can be shared with the pharmaceutical sector, which has expertise in the use of bacteriophage to control microbial populations. Documents produced by colleagues in the field of medical bacteriophage therapy can be adapted to fit food industry requirements.

Conclusion 3: The published literature indicates that there is a potential for bacteriophage to be used to control the level of *L. monocytogenes* on several food types; cooked and processed meats, salad vegetables, vegetables as well as fish and seafood.

Conclusion 4: Evidence suggests that bacteriophage treatments could be capable of delivering effective reductions in the numbers of *Salmonella* spp on raw meat, some fruit and vegetables as well as milk and soft drinks.

Conclusion 5: The literature suggests that bacteriophage treatments may be effective for the reduction of STEC in meat products and fresh produce. Optimisation of the mode of treatment application, bacteriophage concentrations used as well as contact time and temperature is required to ensure that the bacteriophage treatment is fit for purpose.

Conclusion 6: Although bacteriophage shows potential to control *S. aureus* in dairy products, more research is needed to confirm their suitability for use.

Conclusion 7: It is unlikely that a single bacteriophage will be effective against all strains of a particular bacterial species, therefore the use of a cocktail of bacteriophage is recommended to reduce the pathogen/spoilage load on food.

Conclusion 8: The use of bacteriophage to control populations of pathogens or spoilage organisms is influenced by the food itself as well as the storage environment. All bacteriophage treatments should be optimised and validated to ensure that they are fit for purpose.

Conclusion 9: Data from this review indicate that bacteriophage treatments are not a 'magic bullet' and that they should be used in the hurdle approach to food safety.

Conclusion 10: It is recommended that lytic bacteriophage should be used in preference to lysogenic bacteriophage as results suggest that lytic bacteriophage are more effective than lysogenic bacteriophage.

Conclusion 11: There is insufficient published data to assess the full impact of microbial resistance to bacteriophage on the efficacy of bacteriophage to reduce microbial contamination. Therefore more

research is required. It is also recommended that strategies to reduce the potential for formation of bacteriophage resistant mutants,are implemented when bacteriophage treatments are developed.

Conclusion 12: From the evidence to date, the suggested use of bacteriophage would be as a processing aid to reduce the level of microorganisms at a set time point. Further studies are needed to determine the residual activity of bacteriophage on food and their ability to prevent recontamination of food products. In addition, research on the stability of bacteriophage in food relevant conditions (e.g. pH and water activity) is recommended.

Conclusion 13: The literature details methods to ensure safety of bacteriophage for use in the food supply chain, however further work is needed to create common guidelines for safe manufacture and effective application of bacteriophage treatments in the food industry. Amongst the issues to be addressed are i) the impact of bacteriophage infection on toxin formation in known toxin forming bacteria i.e. *S. aureus* and members of *Bacillus* spp. and *Clostridium* spp. ii) the effect of bacteriophage on microflora in the human intestine and in waste effluent from food processing plants and iii) verification that bacteriophage to be used in foods/ food processes do not contain toxin genes or other virulence factors.

Conclusion 14: More research is required to establish the potential of bacteriophage to control spoilage organisms in foods. To ensure that data can be compared between studies it is recommended that the investigations contain the following details

Study characteristics	Requirements
Use of appropriate organisms for food type used	All strains appropriate to food type
Method of inoculation of microorganism into food samples available	Fully available including level of challenge inoculum
The number of organisms used, their source – culture collection or food isolates	More than 3 isolates used. Mixture of culture collection and food isolates
Method of phage application to the food available	Fully available including phage dose
Number of time points tested	Three or more
Number of samples/replicates used	Triplicate samples of one batch or single samples done in triplicate at each time point
Inclusion of relevant controls	Use of appropriate study controls i.e. positive and negative samples for each sample type
Statistical analysis performed on the raw data	Full analysis carried out
Analysis of data performed – log reduction calculated	Log reductions provided

Conclusion 15: Further work is needed to establish the performance of bacteriophage to control microbial contamination in situations that would commonly occur in the food industry. This should include:

i) The use of naturally contaminated foods in pilot trials
ii) An investigation on whether bacteriophage treatments are effective against microorganisms introduced onto the food after the application of bacteriophage into/onto the food

Conclusion 16: The use of bacteriophage lytic enzymes is a promising approach to the control of pathogens and spoilage organisms in foods. One of the key benefits of lytic enzymes is the lack of reliance on virus replication to achieve lysis of the cell. More research is required to determine their fitness for purpose in foods.

GLOSSARY

Colony forming unit	Number of viable microorganisms detected as colonies on the surface of an agar plate
Efficiency of plating (EOP)	The level of the bacteriophage on a given bacterial cell line compared to the maximum theoretical level that can be obtained.
High pressure processing (HPP)	A cold pasteurisation technique by which products, already sealed in their final package, are introduced into a vessel and subjected to a high level of isostatic pressure.
Lysogenic bacteriophage	Bacteriophage that is capable of fusing their genetic material into the host bacterial cell during infection.
Lytic bacteriophage	Bacteriophage capable of producing factors that break down the bacterial cell leading to cell death.
Modified atmosphere packaging (MAP)	Process used to extend the shelf life of food products where air inside food packaging is replaced by gas mixtures through a process of gas flushing.
Multi locus sequence typing (MLST)	Technique used for microbial typing that analyses the nucleic acid sequence of 5 or more target fragments.
Multiplicity of infection (MOI)	Ratio of infective viruses to the host cell
Mutator screen	Assay used to determine the ability of the host to mutate by determining its susceptibility to antibiotics.
One step growth curve	Method used to determine the average number of phage released per host bacterium (burst size).
Pulsed field gel electrophoresis (pfge)	“Fingerprint”-based technique used for typing microorganisms.
Plaque forming units (pfu)	Number of viable virus units detected as clear zones in a lawn of the host culture.
Ready to eat (RTE)	Food that is consumed with no further processing to reduce bacterial load.
Residual activity	Ability to ‘kill’ host bacteria following the initial drop in numbers
Serotype	Group of organisms that share the same antibody profile as determined by a process known as serotyping. Serotyping is a method commonly applied to pathogenic bacteria including *Salmonella* spp and pathogenic *E. coli*.
Shiga toxin producing *E. coli* (STEC)	A group of *E. coli* that possess 2 factors: shiga toxin producing genes (stx); and attachment genes such as initmin (eae). STEC are known to be highly pathogenic and are capable of causing severe disease in humans. They are also known as verocytotoxin producing *E. coli* (VTEC)
Temperate bacteriophage	Bacteriophage that are capable of fusing their genetic material into the host bacterial cell during infection.
Transduction	Process where bacterial DNA can be transferred between cells in the viral capsid.
Ultra-heat treatment (UHT)	High temperature heat process used as an equivalent treatment to pasteurisation.
Whole genome sequencing (wgs)	Technique used to determine the nucleic acid sequence of the complete genetic material of an organism.

1 INTRODUCTION

This project was conducted in response to a call by the FSA for a critical review of the potential use of bacteriophage on foods to act as an additional control for food pathogens and spoilage organisms within the supply chain. The scope of the project was limited to foods, as previous work on the use of bacteriophage in live animals has already been commissioned.

The literature reports that approximately 1 million people suffer from food borne illness in the UK each year, and of these 20,000 receive hospital treatment as a result of that illness (FSA 2011). It is estimated that the economic burden of food borne disease in the UK is just under £1.5bn (FSA 2014). Reports indicate that the key causative agents of food-related gastroenteritis in the UK are bacterial pathogens including *Campylobacter*, *Salmonella* and shiga toxin producing *E. coli* (STEC). As part of the FSA strategy to reduce levels of foodborne illness in the UK, horizon scanning for novel approaches to control food safety risks has been highlighted as a key approach. In addition, there is also a need to reduce the level of food wastage as a result of food spoilage. Studies carried out on food waste in the UK in 2013 revealed that approximately 15 million tonnes of food waste was generated across all of the food sectors; household, manufacturing, retail and wholesale hospitality and food service, food waste in litter and pre farm gate. Of the food disposed of in 2012, it is estimated that around 21% of the food waste arose from food spoilage (Wrap 2015) **http://www.wrap.org.uk/sites/files/wrap/UK%20Estimates%20October%2015%20%28FINAL%290.pdf**.

There have been an increasing number of papers published on the application of bacteriophage for the control of bacterial pathogens on foods. Bacteriophage are viruses that can only infect bacteria and their name was derived from the Greek 'bacteria eaters'. All bacteriophage are obligate parasites and require a host (bacteria) to enable them to replicate. Bacteriophage are abundant in nature and have been isolated from a range of sources including saltwater, freshwater, soil, plants and animals as well as the human skin, gastrointestinal tract and genitourinary tract (Andreoletti et al. 2009). Bacteriophage have two predominant lifecycles; those that follow an immediate lytic cycle (lytic or obligately lytic bacteriophage) where the bacterial cell is fatally ruptured when the viruses are released from the host cell, and those which do not immediately lyse their host (temperate or lysogenic bacteriophage) but insert their DNA into the host genome where it can be replicated until extrinsic factors trigger entry into the lytic cycle. Other rare bacteriophage lifecycles exist, such as secretion and pseudolysogeny, but these bacteriophage types are not considered in this review.

The key applications of bacteriophage in the food industry that have been reported are: reduction of pathogen colonisation in animals during primary production; reduction of the colonisation on food's surface; prevention of pathogen contamination and growth on foods during shelf life; and the disinfection of contact surfaces (Sillankorva et al. 2012). These authors stated that bacteriophage offer several benefits as biocontrol agents including:

- High specificity due to intimate binding with external receptors on the host cell;
- Low doses required due to self-replicating and self-limiting properties of the bacteriophage;
- Continuous adaptation of the bacteriophage to overcome potential bacterial resistance;
- Low inherent toxicity to humans;
- Ability to be produced cost effectively;
- Thought to withstand environmental conditions in the food production environment; and
- Have proven track record of extending product shelf life.

This project provides a structured critical review of the data currently available on the application of bacteriophage to foods in relation to their efficacy, specificity, safety and any potential issues associated with their use. This data is currently unavailable in a collated format, and will provide a useful resource for industry, competent authorities and academia alike. The review was carried out by a team of information specialists and specific experts in the fields of bacteriophage as well as the evaluation of decontamination and preservation protocols. Initially, a survey of the current applications of bacteriophage in the food industry was performed to collate information in 3 areas:

- Current global use of bacteriophage within the food industry to control microorganisms on food products;
- Published controls for the use of bacteriophage within the food industry; and
- Safety considerations and recommendations on the use of bacteriophage as biocontrol agents on foods.

As a second part of the project, a systematic and critical review was done to gather data on the current research on the application of bacteriophage as biocontrol agents on foods. Publications for the review were collated using a defined set of search terms and reviewed (by 2 of the scientific experts), according to set inclusion/exclusion criteria based on data quality. All publications were sent out using a blind approach with author and journal details removed to eliminate potential bias.

Data gathered in the review was summarised in evidence tables for key food pathogens (*Salmonella* spp., *Campylobacter* spp., *L. monocytogenes*, pathogenic *E. coli* and *Staphylococcus aureus*), 5 key groups of spoilage organisms (Gram negative rods, the Enterics, Gram positive spore formers, Gram positive non spore formers and lactic acid bacteria). The evidence tables were used to assess the efficacy of bacteriophage to reduce pathogen loads on foods. Scientific conclusions were drawn from the data to address the following points:

- Food products compatible with bacteriophage treatments;
- The efficacy and specify of bacteriophage;
- The potential for their use to protect against recontamination;
- The safety of bacteriophage on foods and in food production;
- Limitations on use of bacteriophage;
- Any potential risks or issues with bacteriophage use such as the possible emergence of bacterial resistance to treatments with bacteriophage;
- Whether bacteriophage is effective at point of application only (used as a processing aid) or whether it remains active over shelf life (i.e. considered as an additive).

2 CURRENT USE OF BACTERIOPHAGE

The initial objective of the project was to collect evidence on the current application of bacteriophage across the world to control the bacterial load on food products with specific reference to the following points:

- Current global use of bacteriophage within the food industry to control microorganisms on food products;
- Published controls for the use of bacteriophage within the food industry;
- Safety considerations and recommendations on the use of bacteriophage as biocontrol agents on foods.

To collate the relevant information, literature searches were conducted on FSTA from 2009 onwards. A limit on the search date was applied to reduce duplication of references cited in the EFSA report on the use and mode of action of bacteriophage in food production (EFSA 2009). The literature search used 3 terms:

- Bacteriophage for biocontrol of foodborne pathogens;
- Safety of bacteriophage on foods/ in food production; and
- Bacteriophage and foods controls/guidelines/recommendations/risk assessments (and thesaurus term ‘’food’’).

In addition, patent websites were interrogated to highlight web pages of bacteriophage producers and food research organisations/competent authorities/advisory bodies that can be accessed to collate information on the current use of bacteriophage in industry. Full details of the searches are outlined in the methodology section (Section 3.1 for reference).

Three hundred and forty two (342) papers were gathered during the initial search and these were supplied to the project manager in abstract format. The abstracts were reviewed for relevance and references containing details on the use of bacteriophage to control food microflora were requested for inclusion in the initial literature review carried out in section 2. Web searches carried out in this aspect of the project provided information on the current use of bacteriophage in the food industry. A review of the search on the use and control of bacteriophage in the global food industry revealed that limited data was available. To supplement the information gathered, the expert reviewers were asked to forward references relating to the safety of bacteriophage as biocontrol agents in foods and recommended controls on their use.

2.1 Commercial use – global

The first bacteriophage-based product to receive approval for use in the food industry was Agriphage (Omnilytics Inc.), which was developed in the USA as a treatment to control bacterial spot disease on agricultural crops (US Environ. Prot. Agency 2005). Since then approval has been obtained for several food applications. LMP-102, later named Listshield™ (Intralytix Inc.), was the first bacteriophage product used to control the food borne pathogen *L. monocytogenes*. It was approved by the USA Food and Drug Administration (FDA) for ready to eat (RTE) meat and poultry products in the USA as a food additive (Bren, 2007, Anon 2016). Additional products for the biocontrol of *L. monocytogenes* have been granted ‘Generally Regarded As Safe’ (GRAS) status for use in the food industry. These include

Listex P100 (EBI Food Safety), as a processing aid for all foods that are susceptible to contamination by this pathogen (Hagens and Offerhaus 2008). Listex™ has subsequently been reported to have gained approval for use as a clean label processing aid in countries including the USA, Canada, Australia, New Zeeland, Switzerland, Israel, Norway and The Netherlands (**http://www.listex.eu/regulatory/**) Furthermore Listex™ has been certified organic and complies with the requirements of USDA's National Organic Program. More recently Listex™ has been approved by Food Standards Australia and New Zealand (Endersen et al. 2014).
Other products targeting different pathogens have also obtained approval for use in the US. Following initial approval for the decontamination of animals pre-slaughter, EcoShield (Intralytix Inc) was approved for application on red meat being used for hamburger production in 2011 to control *E. coli* O157:H7 (Sillankova et al. 2012). Salmofresh™ (which targets *Salmonella enterica*) was approved in 2013 for direct application onto poultry, fish and shellfish as well as fresh and processed fruits and vegetables (**http://www.intralytix.com**). In addition Salmofresh™ has approval for use as a processing aid in the production of poultry products.

Several patents have been submitted for the biocontrol of microorganisms, however the majority of these have focussed on clinical applications. The current project identified 18 patents that had a relevance to the food industry. Interestingly there were also patent submissions specific for the control of organisms covered in this review in animal feed and food processing surfaces including *Salmonella* and *Listeria monocytogenes*.

Arguably, the greatest use of bacteriophages for controlling microbes on a volume basis in the food industry globally is for pre-harvest interventions. There are several examples of this, including Finalyse™ (marketed by Elanco, developed by Omnilytics Inc.) and STECleanz™ (ESR Ltd.), which both target Shiga-toxigenic *Escherichia coli* on animal hides to reduce the load on red meat, and ArmamentTM (Intralytix) which targets *Salmonella* on chicken feathers to reduce the load on poultry products. The use of bacteriophage-based pre-harvest interventions was excluded from this study as it was considered out of scope, but is noted here to provide a context for the use of bacteriophages in the whole farm to fork food production system.

Key finding 1: Generally Regarded As Safe (GRAS) approved, commercially produced bacteriophage are being used as food additives in the US and as processing aids in parts of Europe. The products are being used to reduce the pathogen load of *L. monocytogenes*, *Salmonella enterica* and *E. coli* O157:H7 on a range of named food types; poultry, red meat, RTE foods, fish, shellfish as well as processed fruits and vegetables.

Key finding 2: The major use of commercially produced bacteriophage in the food industry to date is in the pre-harvest control of foodborne pathogens including STEC and *Salmonella*.

2.2 Perceived risks/ safety recommendations

In the USA commercially available bacteriophage treatments must be certified by the Food and Drug Administration (FDA) with the status Generally Regarded As Safe (GRAS). For a substance to qualify for GRAS, scientific evidence needs to be collated to demonstrate its safety which has been reviewed and accepted by qualified experts in the field. By gaining approval, the competent scientists must have reasonable certainty that the substance is not harmful under its intended condition of use (21 CFR section 170.3(i)). The experts involved in the approval process must reach a consensus opinion using

a clearly defined decision-making process and all the information used in the GRAS approval must be generally available (21 CFR section 170.30(a)-(c)).

However, there are still issues to be addressed before achieving the routine use of bacteriophage as biocontrol agents on foods. One of the main areas of consideration is the formulation of guidelines for the safe and effective use of bacteriophage in food production. Hagens and Loessner in 2010 published a list of desirable characteristics for bacteriophage to be used as biocontrol agents:

- Possesses a broad host range to ensure it will be effective against several strains of the target species or genus;
- The bacteriophage should not incorporate into the host genome (i.e. strictly lytic) during the infection cycle;
- Capable of propagation in a non-pathogenic host;
- Availability of the complete genome sequence of the bacteriophage being used;
- Bacteriophage infection cycle should not involve the incorporation of non-bacteriophage DNA into the bacteriophage genome (transduction);
- Lack genes encoding pathogenic properties or potentially allergenic proteins;
- Show no adverse reactions in animal feeding trials;
- Bacteriophage-based products should achieve GRAS status or other regulatory approval; and
- Be amenable to scale up for commercial production

Safety concerns relating to the use of bacteriophage in the food industry fall into 3 main areas as listed in the EFSA evaluation of the bacteriophage product Listex P100 for the removal of *L. monocytogenes* contamination on raw fish:

- Toxicological safety;
- Emergence of reduced susceptibility to biocides/antimicrobials linked to the use of the product;
- Risk of product release into the environment.

The toxicological safety takes into account the chemical reactivity of the bacteriophage product and should include the formulation, the bacteriophage itself and the bacteriophage production process. Many bacteriophages are composed of a DNA or RNA molecule surrounded by a protein coat (capsid), which would break down into amino acids and nucleic acids. Both amino acids and nucleic acids are naturally found in foods and it has been proposed that these would not increase the risk to consumers (Andreoletti, et al. 2009). In addition, it is thought that the level of bacteriophage reduces significantly in humans when ingested as a result of two key processes: passage through the stomach which is at high acidity; and the breakdown of bacteriophage by digestive enzymes that degrade the bacteriophage capsid.

Further points to consider in relation to consumer safety would be the potential for the bacteriophage-based product to produce compounds that could lead to an allergic reaction in sensitive populations. Searches should be carried out to check for the presence of structures that could cause an allergic response. If potential allergic regions are highlighted, then further consultation with experts in the field of Dietric products, nutrition and allergies should be sought. To back up the conclusions by experts in nutrition, an oral toxicity study could be performed to verify opinion. The oral toxicity study should be carried out following a recognised code of practice such as GLP to ensure that the evaluation is scientifically robust. A few oral toxicity studies involving human volunteers have been published (McCallin et al. 2013, Sarker et al. 2012) as well as some in rats (Carlton et al. 2005, Kang et al. 2013).

The generation of toxic compounds during commercial bacteriophage production should also be avoided. Where possible it would be recommended that no animal products are used in the growth medium for the bacterial host. Due to the bacteriophage specificity the production of bacteriophage products could often involve the use of pathogenic bacteria as hosts. To reduce the risk to consumers it is essential that bacteriophage preparations are free from pathogens themselves as well as biological material that could cause illness. High temperature treatment used to remove host cells could also be another possible route to generate toxic compounds (EFSA 2012). An alternative to heat treatment would be filtration with a filter of an appropriate pore size to eliminate host organisms, or separation by affinity chromatography.

In addition to the production process, the characteristics of the bacteriophage itself should be analysed to check that the bacteriophage nuelcic acid sequence does not contain:

i) Virulence, toxin, or antibiotic resistance genes, or
ii) Genes that may cause mutation of the bacteriophage, or
iii) Genes enabling the bacteriophage to integrate its genetic material into the host (Strauch et al. 2007).

One possible approach to reduce the likelihood of the transfer of undesirable genes into the final bacteriophage product would be to use a non-pathogenic bacterial isolate for bacteriophage production, e.g. *Listeria innocua* for the production of *L. monocytogenes* bacteriophage. Another issue to take into account is the potential for the bacteriophage to infect plant, human or animal cells. One of the characteristics of bacteriophage is that they are highly host specific and are therefore thought to be harmless to humans, animals and plants (Sillankorva et al. 2012).

Another point for consideration is the potential for the emergence (within the host bacteria) of resistance to biocides or key therapeutic antimicrobials used to treat bacterial infections. Analysis of the bacteriophage sequence would reveal the presence of potential genes that are associated with antimicrobial resistance. Other bacteriophage characteristics that could cause issues would be the ability to incorporate host genetic material during a process known as transduction. It is known that transduction can mobilise plasmids that confer resistance to antibiotics in Staphylococci (Firth and Skurray, 2000) and Streptococci (Caparon 2000). The literature has reported the transfer of resistance to tetracycline, chloramphenicol, macrolides, lincomycin, and clindamycin (Ubukara et al. 1975) and streptomycin (Hyder and Streitfield, 1978), probably via generalised transduction of non-bacteriophage-encoded resistance genes (Wagner and Waldor 2002). In addition, the efficacy of the bacteriophage treatment should be regularly monitored to check for the development of natural resistance of host bacteria to the bacteriophage treatment.

The optimal application time of the bacteriophage product during food production should also be considered. To ensure an effective decontamination it is essential that the bacteriophage is spread evenly over the food surface (usually by dipping or spraying). In their application dossier to EFSA, the applicant for the Listex P100 also acknowledged that the bacteriophage treatment should be carried out as early in the process as is feasible. Early application of the bacteriophage will ensure that any potential contamination with *Listeria monocytogenes* is not spread throughout the processing environment and potentially act as a source of recontamination in the future (Andreoletti, et al. 2009). Following the completion of an appropriate contact time, consideration must be given to the fate of the bacteriophage post treatment. In some cases the addition of a neutralisation removal/recycling step may be required, however this will be dependent upon the bacteriophage treatment being applied. The need for a neutralisation removal/recycling step will also be dependent on the stability and type of

bacteriophage being used. It has been proposed that lytic bacteriophage (which rapidly breaks down to form components typically found in food), should not be regarded as being hazardous for humans (EFSA, 2009).

The risks associated with product release into the environment have to be assessed. Many of the aspects of product safety would be addressed in relation to consumer safety including the chemical reactivity of the bacteriophage product and formulation. A risk assessment of product release should be done and should take into account the likely constituents of the waste, as well as the potential dosage which would be released and the location of the discharge. Due to the lack of metabolism within bacteriophage, it is possible that they are able to persist in the environment for relatively long periods of time. In reality, the dependence of bacteriophage on divalent cations and their susceptibility to temperature shifts, UV light and microbial proteases (produced by the environmental bacteria) results in rapid inactivation and break down. After inactivation, the bacteriophages are degraded into amino acids and nucleosides that are not known to represent a risk for the environment (Carlton et al. 2005).

Residual bacteriophage in the product may also impact on the detection of microorganisms in a food manufacturer's monitoring programmes, particularly for the presence of pathogens. Residual activity of bacteriophage during testing could potentially result in a false negative result, or an underestimation of the bacterial load in the product. It has been reported that bacteriophage can persist in food products for up to 2 weeks (Anon, 2012). Investigations into the impact of bacteriophage on the detection and enumeration of the pathogen *L. monocytogenes* have been carried out. Some studies included the use of an additional centrifugation step to separate the bacteriophage from the host bacteria before analysis (Bigot at al 2011, Soni and Nannapeni 2010). This step though is not consistently carried out across all work done (Anon, 2012). Analysis of the data from challenge studies concluded that the short time taken to plate out would not be sufficient for the bacteriophage to infect host cells. Furthermore, the use of a diluent for sample preparation and serial dilution reduces the concentration of both bacteriophage and bacteria thus reducing the likelihood of them being in contact with each other to a minimum (Food Standards Australia and New Zealand, 2012). Studies into the impact of the presence of bacteriophage (P100) in food on the detection of *L. monocytogenes* revealed that the *Listeria* could be detected in spiked cheese after storage for 21 days (Schellekens et al. 2007), using a sample enrichment method.

The potential of bacteriophage therapy is being explored in many fields as well as food, and so it may be useful to adopt some of the same regulatory approaches used in these fields. The longest application of bacteriophage therapy has been in the clinical setting where they were first used as therapeutic agents in humans in 1919 (Summers, 1999). Bacteriophage therapeutic products have already been marketed in Eastern European countries including the Republic of Georgia (Parracho et al. 2012). To date, there are no published guidelines for bacteriophage therapeutic products either in Europe or in the US (Parracho et al. 2012). In Europe the development of bacteriophage-based medicinal products is controlled by Good Manufacturing Practice (GMP), which is a regulation that is applied to all medicinal products and not just bacteriophage-based treatments. If bacteriophage is to be used in food products, their production should be overseen by a safety management system based on Hazard Analysis Critical Control Points (HACCP) principles. The implementation of a HACCP-based approach will enable the producer to comply with EU Regulation (EC) 852/2005, which is a requirement for all food business operators along the food chain.

In 2015, Pirnay et al. published expert consensus quality and safety requirements for medicinal bacteriophage products that could be adopted for bacteriophage products used in the food industry.

The Pirnay publication recommended that the production of intermediate, bulk of finished bacteriophage products should be carried out in a controlled environment with 9 key requirements:

- Production should be done in a specified air quality to reduce the risk of airborne product contamination. The air quality needed should be validated as fit for purpose and should be regularly monitored when in use;
- The level of containment required for the production facility is appropriate for the organism being used as the bacteriophage host;
- Appropriate equipment is used that has been designed to minimise hazards to the production staff, users of the product, and consumers of the end food product where the bacteriophage based treatment has been applied;
- All equipment should be maintained and regularly inspected according to the manufacturer's instructions. Records must be kept of the maintenance, servicing, cleaning and disinfection carried out at regular intervals;
- The production process should be validated to verify fitness for purpose and documented within the business management system/quality policy as an SOP. The SOP should also contain/reference:

  - Specifications of critical supplies;
  - The critical control steps within the process along with details of appropriate monitoring procedures, alert/alarm systems and corrective actions;
  - Details of analysis required to ensure product safety and quality.

- The implementation of bacteria and bacteriophage bank systems which should ideally include master and working stock banks. All bacteria and bacteriophage should be characterised to identify relevant genotypic and phenotypic markers which can be used to verify strain authenticity, and to determine purity of bacterial stocks and viability of host stocks;
- Well defined quality control and quality assurance are in place for bacterial host stocks, master bacteriophage stocks, working bacteriophage stocks and finished product.

An example of the suggested criteria for QA/QC is displayed in the following Tables (1-4):

Table 1: Guidance Quality Control and Quality Assurance criteria for stocks of host bacteria used in the bacteriophage production process sourced from Pirnay et al. 2015

Characteristics	Control test	Limits of acceptance	Recommended test procedure
Strain origin	Documented pedigree/history/hazard group/pathogenicity	Of known origin	Screen of strain consignment letters/documentation, laboratory records and scientific literature
Strain identity of working stock	Authentication of isolate and species and strain profile	Confirmation that species identification and strain profile match records held for isolate in use	State of the art identification methodology. Highly discriminatory molecular/genomic typing methods e.g. MLST, PFGE, WGS
Ideally non lysogenic strain, or at least one that contains as few prophage or possible mobile genetic elements as possible	In vitro induction tests that expose cells to environmental stress such as UV	As few temperate bacteriophage produced as possible during induction test	In vitro induction screen using mitocycin C (Burlage et al. 1998) or ultra violet UV radiation
	Host genome screen for bacteriophage or bacteriophage like elements	As few prophage or bacteriophage like elements in the genome sequence as possible	State of art DNA sequencing and bioinformatic analysis
Lack of ability to mutate	Screen for mutator capability	No mutants produced	State of art mutator screen e.g. fosfomycin and rifampicin disk diffusion tests (Galan et al. 2004)
Validated production method/storage parameters	Monitoring of storage parameters	Process dependant	Appropriate temperature monitoring devices

Table 2: Guidance Quality Control and Quality Assurance criteria for bacteriophage seed stocks used in the bacteriophage production process sourced from Pirnay et al. 2015

Characteristics	Control test	Limits of acceptance	Recommended test procedure
Strain origin	Documentation of bacteriophage pedigree/history source	Of known origin	Screen of strain consignment letters/documentation, laboratory records and scientific literature
Strain identity	Authentication of the family, genus, species, strain profile, biology and morphology of the bacteriophage stock	Confirmation that the family, genus, species, strain profile, biology and morphology bacteriophage stocks matches the records held	➢ State of the art methods for: ➢ DNA/RNA sequence analysis ➢ Highly discriminatory typing techniques ➢ Classification according to the International Committee on the taxonomy of viruses (ICTV) ➢ Electron microscopy (optional) ➢ One step growth curve (Kutter and Sulakvelidze, 2005)
Absence of potentially damaging characteristics conferring on the host bacteria i.e. virulence, toxicity, ability to acquire genetic material (lysogeny) or antibiotic resistance	Analysis of bacteriophage genome for harmful determinants	Absence of potentially damaging determinants listed in column 1	State of the art RNA/DNA sequence and genomic analysis

Characteristics	Control test	Limits of acceptance	Recommended test procedure
No ability to transfer genetic material from one bacteria to another i.e. non transducing (optional)	Screen for general transduction	Does not incorporate any of the host genetic material into bacteriophage progeny	Recognised Transduction assay (Petty *et al.* 2006)
In vitro efficacy	Determination of host range	Broad host range as possible	Plating bacteriophage with target bacteria using soft overlay method
	Stability of lysis	Lysis stable for 24-48h in broth culture	Applemans method (Applemans, 1921)
	Bacteriophage activity under conditions similar to those being used in the field	Pass of set EOP criteria	Standardised efficiency of plating (EOP) method
	Frequency of bacteriophage resistant bacteria	Low frequency of emergence of resistance	Method documented in Adams 1959
Validated production method/storage parameters	Monitoring of storage parameters	Process dependant	Appropriate temperature monitoring devices

Table 3: Guidance Quality Control and Quality Assurance criteria for bacteriophage working stocks used in the bacteriophage production process sourced from Pirnay et al. 2015

Characteristics	Control test	Limits of acceptance	Recommended test procedure
Pre-determined bacteriophage load during production	Bacteriophage enumeration/titration	Variable but will typically be 10^8 to 10^{10} plaque forming units per ml (pfu/ml)	Standardised soft overlay method
Microbial contamination	Sterility	Absence of microorganisms	Membrane filtration using standardised methods
		Absence of pathogens	State of the art microbiological methods
Toxicity	Determination bacterial toxin presence	Toxin specific – dependant on sensitivity of available assays	Specific toxin assays specific to the organism where required
Bacterial DNA contamination	Screen for potentially damaging host bacterial DNA	Absence of potentially damaging genetic determinants known to be present in the target host bacterium	Recognised method for determination of bacterial DNA sequences e.g. PCR
Acidity/ basicity (pH)	Check pH value is in optimum working range	Variable but typically 6.5-7.5	Standardised pH test
Purity	Clarity of bacteriophage solution	Absence of visible particles	Recognised method for the detection of particulates
Validated production method/storage parameters	Monitoring of storage parameters	Variable but usually 2-8°C	Appropriate temperature monitoring devices

Table 4: Guidance Quality Control and Quality Assurance criteria for finished bacteriophage based products sourced from Pirnay et al. 2015

Characteristics	Control test	Limits of acceptance	Recommended test procedure
Validated production method/storage parameters	Monitoring of storage parameters	Variable but usually 2-8°C	Appropriate temperature monitoring devices

- Documented checks required to determine shelf life of working stocks, solutions and finished product;
- An active surveillance of the literature to flag up issues associated with adverse events and reactions associated with the use of bacteriophage therapy via a centralised reporting system.

Further recommendations would relate to the labelling of the packaging of the final product. It is suggested that the label or accompanying documentation should contain a description of the product, date of production, instructions for storage and opening of the product, use by date as well as details of the disposal of unused product.

Key finding 3: Although bacteriophage are being approved for use in the food industry one of the main barriers to their wider use is the formulation of guidelines for their safe and effective use. The three key safety concerns are: toxicological safety; emergence of resistance to biocide and antimicrobials linked to the use of bacteriophage; and the risk of bacteriophage release into the environment.

Key finding 4: Research on the use of bacteriophage in the clinical sector is available, however official guidelines/regulations are yet to be published for bacteriophage therapeutic products in the US or Europe.

Key finding 5: A peer reviewed expert consensus on the quality and safety requirements is available for therapeutic products which could be modified for use in the food industry.

2.3 Controls on use of bacteriophage in the food industry

To the author's knowledge, no controls specific to the use bacteriophage treatment on foods have been published in countries where bacteriophage is currently being used. There are however certain circumstances where current legislation would apply to the use of bacteriophage on foods in the UK and Europe. Approval of bacteriophage treatment is required under hygiene regulation (EC) No 853/2004) if bacteriophage treatments are to be applied to the surface of meat or fish to remove bacterial contamination. Guidance has been published by EFSA (EFSA, 2010) on the submission of data for the evaluation of substances (including bacteriophage) for microbial surface contamination. The guidance outlines a documented procedure to follow for authorisation of surface decontamination treatments.

The use of bacteriophage is governed by general legislation documented in the Food Additive Regulation (EC) No 1333/2008 . The regulation defines a food additive as '*any substance not normally consumed as food in itself and not normally used as a characteristic ingredient of food*

whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by- products becoming directly or indirectly a components of such foods'. This definition applies to bacteriophage that remains active on the food product and is capable of destroying their target organism (pathogen/spoilage organism) over its shelf life.

Bacteriophage that breaks down rapidly/loses antimicrobial activity over product life however will be classified as a processing aid and is also governed by general legislation in the Food Additive Regulation (EC) No 1333/2008 . A processing aid is defined as ' any substance which:(i) is not consumed as a food by itself; (ii) is intentionally used in the processing of raw materials, foods or their ingredients, to fulfil a certain technological purpose during treatment or processing; and (iii) may result in the unintentional but technically unavoidable presence in the final product of residues of the substance or its derivatives provided they do not present any health risk and do not have any technological effect on the final product.' Processing aids are controlled by national regulations across the EU and in the UK there is no current legislation specific to bacteriophage treatments in place (FSA 2014). All foods produced using processing aids would however need to comply with general food law which states that food must be safe for human consumption.

There is a paucity of information on the activity of bacteriophage during storage, and evidence on the multiplication of bacteriophage in a product over life has been reported to be inconclusive (Leverentz et al. 2003). Indeed, it is likely that bacteriophage activity will differ for each food type. For example, as bacteriophages rely on passive diffusion, it may be that liquid foods or foods with high water activity will permit continuous circulation of bacteriophages in/on the product and could therefore potentially protect against recontamination events. Whereas some components of foods are known to inactivate bacteriophages by binding to the virion, such as milk, whey proteins (Gill et al. 2006) and components of black tea (Siqueira et al. 2006). Therefore the area of residual bacteriophage activity over product shelf-life is a research gap which needs to be addressed.

Key finding 6: At present there are no specific controls in place that are specific to the use of bacteriophage treatments on foods. There are however several regulations that must be complied with when using bacteriophage treatments in/on food products across Europe:

i) Hygiene regulation (EC) No 853/2004 applicable to bacteriophage treatments that are to be applied to the surface of meat or fish to remove bacterial contamination;

ii) Food Additive Regulation (EC) No 1333/2008 detailing controls specific to food additives and food processing aids; and

iii) General food law which states that food must be safe for human consumption

3 METHODOLOGY FOR CRITICAL REVIEW

3.1 Definition of literature search criteria

- Definition of search terms

An initial general search for bacteriophage*/phage* was conducted to ascertain the volume of information available with the following results:

Database	Number of results
Food Science and Technology Abstracts (FSTA) 1969-2015	4520 (open term), 2078 (thesaurus term)
Foodline: science (1972-2015)	2314 (open term), 1204 (thesaurus term)
Current Contents (1998- 2015) (Modules including Life Sciences and Agriculture, biology and environmental sciences)	26,595 (open term), 746 (research area: food science technology)
PubMed	1346 (with “food” and as MeSH terms)

Information collated from the initial general search was then used to refine future searches to optimise the data gathered in the critical review.

Trial searches were then conducted on FSTA from 2009 onwards using search terms based on the original proposal. A limit on the search date was used to prevent the duplication of references cited in the EFSA report on the use and mode of action of bacteriophage in food production (EFSA 2009). The purpose of the search was two-fold:

i) To gather the information on the current use of bacteriophage in the food industry; and
ii) To enable the review team to define the search terms to be used in the critical review.

The initial search was carried out using a defined set of search terms detailed in Table 5.

Table 5: Details of search terms used for the trial search

Bacteriophage* OR phage* AND DE/foods combined with:

Terms	Results
Control* OR guideline* OR recommend* OR risk assess* OR code*	140
Pathogen*	140
Biocontrol*	26
Safe*	331
Biocontrol* AND pathogen*	16
Total unique references	**342**

The resulting 342 abstracts were scanned by the Project Manager and 68 full text articles were selected to enable the project team to clarify the exact terms and combinations thereof for the main search. In addition, data gathered in the initial search was used to obtain information on the current

global use of bacteriophage and to establish if guidelines had been published on the safety and use of bacteriophage in the food industry.

Following the initial searches, adjustments were made to the search terms according to the specific search facilities of each database, e.g. availability of thesauri and facilities such as truncation (indicated by *). The search terms used were based on the organism selected for inclusion and were combinations of bacteriophage*, phage*, biocontrol*, food*, and lysin. On FSTA, thesauri terms "analytical techniques, typing and ribotyping" were excluded to further limit the search.

Individual searches were carried out for each of the selected pathogens and representative spoilage organisms chosen. Five food pathogens were taken forward into the review and these were the 5 major food pathogens in the UK namely: *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, shiga toxin producing E. coli (STEC) and *Staphylococcus aureus*.

The current literature identifies 5 recognised groups of spoilage organisms (Gram negative rods, the Enterics, Gram positive spore formers, Gram positive non spore formers and lactic acid bacteria). Representative organisms were selected for each of the spoilage groups as listed in Table 6.

Table 6: List of organisms selected as being representative of the 5 recognised spoilage groups

Spoilage group	Representative organisms
Gram negative rods	*Pseudomonas* spp
Enterics	*E. coli*, *Citrobacter* and *Hafnia*
Gram positive spore forming bacteria	*Bacillus* and *Clostridium*
Gram positive non spore forming bacteria	*Micrococcus* and *Brocothrix*
Lactic acid bacteria	Lactic acid bacteria

3.2 Data sources

- Databases

Literature searches were conducted using the following databases:

- Food Science and Technology Abstracts (FSTA) (2009-2015 (June))
- Foodline: science (2009-2015 (June))

Reference Manager (RM) version 10 was used to store the results from FSTA and Foodline: science (citations and abstracts). All results were cross checked and duplicates removed. The search result numbers from the databases are given in Table 7.

Table 7: Food Science and Technology Abstracts/Foodline: science (2009-2015) searches

Pathogen	Total number of unique articles
Bacillus (BA)	58
Brochothrix (BR)	2
Campylobacter (CA)	36
Citrobacter (enteric) (CI)	6
Clostridium (CL)	25
Escherichia coli (enteric) (ES)	33
Hafnia (enteric) (HA)	5
Lactic acid bacteria (LA)	53
Listeria monocytogenes (LI)	115
Micrococcus (MI)	1
Pseudomonas (PS)	30
Salmonella (SA)	93
Staphylococcus aureus (ST)	70
STEC (STE)	94

A current awareness search was also conducted on FSTA for bacteriophage* OR phage* to identify additional articles being published during the review time period. This was closed at the end of the article review period.

Due to the large number of references for *Listeria monocytogenes*, *Salmonella* and STEC the Project Manager screened titles and abstracts and selected those for sending to the reviewers. The criteria used were:

- Reference to one of the organisms included in the critical review;
- Involve the use of bacteriophage as a biocontrol agent;
- Abstract in English.

Further checks were done on the references gathered for the *Escherichia coli* references to filter out STEC into the correct organism group.

- Patent search

A patent search was conducted on Thomson Innovation (2009-2015 (August)) searching collections:

US Granted, Australian Innovation, Canadian Applications, US Applications, Australian Granted, French Granted, French Applications, European Granted, Australian Applications, German Utility Models, European Applications, British Applications, British Granted, German Granted, WIPO Applications, Canadian Granted, German Applications, Chinese Utility Models, Indonesian Simple, Korean Utility Models, Singaporean Applications, Chinese Granted, Indonesian Applications, Korean Granted/Examined, Thai Granted/Examined, Chinese Applications, Japanese Utility Models, Korean Applications, Vietnamese Granted, Indian Granted, Japanese Granted, Malaysian Granted, Vietnamese Applications, Indian Applications, Japanese Applications, Singaporean Granted, DWPI, Other Authorities.

The search terms used (in title/abstract) were:

CTB=(bacteriophage* OR phage*) AND CTB=(campylobacter OR listeria ADJ monocytogenes OR Salmonella OR Escherichia ADJ coli OR Staphylococcus ADJ aureus OR citrobacter OR hafnia OR pseudomonas OR brochothrix OR micrococcus OR clostridium OR bacillus OR lactic ADJ acid ADJ bacteria).

There were 2768 results which were then limited by the following classifications:

- A01 (agriculture, forestry, animal husbandry, hunting, trapping, fishing);
- A21 (baking, edible doughs);
- A22 (butchering, meat treatment, processing poultry or fish);
- A23 (foods or foodstuffs; their treatment, not covered by other classes);
- C12 (biochemistry, beer, spirits, wine, vinegar, microbiology, enzymology, mutation or genetic engineering);
- C13 (sugar industry).

This resulted in 930 patents. The titles and short abstracts of these were scanned by the Project Manager who selected 104 to obtain detailed abstracts. Duplicates with the FSTA and Foodline: science searches were removed. Full text was then obtained for 29, of which 18 were placed into relevant organism group and sent out to two reviewers. The criteria used for inclusion in the review were as follows:

- Reference to one of the organisms included in the critical review;
- Involve the use of phage as a biocontrol agent in food;
- Published in English

- Organisation/Grey literature and Thesis/Dissertation websites

Websites were searched either using the sites search facility where available or Google advanced search option limiting to the appropriate website address. Search terms “bacteriophage” or “phage” were used and results were limited to 2009-2015.

The websites agreed at the project meeting on 15 April were searched:

CSIRO
European Food Safety Authority (EFSA)
Food Standards Australia and New Zealand
Health Canada
Institute of Environmental Science and Research (IESR)
Institute of Food Research (IFR)
United States Department of Agriculture online safety resources including the Food Safety Research Information
Open Grey (http://www.opengrey.eu/)

The following company websites were identified from the textbook ‘Natural antimicrobials in food safety and quality’, Rai and M Chikindas (editors), CABI, 2011, ISBN 9781845937690 and Annual Reviews in Food Science and Technology, 2014, pp 327-349.

Table 8: Company websites checked

Company	Website
AmpliPhi Biosciences Corporation	http://www.ampliphibio.com
BigDNA	www.bigdna.com
Biocontrol	www.biocontrol-ltd.com
Biophage Pharma Inc	www.biophagepharma.net
Biophage Pharmaceuticals	www.biopharmservices.com
Biopharm Ltd	No website identified
Blaze Venture Technologies	No website identified
CJ CheilJedang Corporation	http://www.cjingredient.com/index.asp
EBI Food Safety	www.ebifoodsafety.com
Elanco Food Solutions	http://www.elanco.com
Gangagen Inc	www.gangagen.com
Hexal Genentech	www.hexal-gentech.com
Innophage	www.innophage.com
Intralytix	www.intralytix.com
JSC Biochimpharm	www.biochimpharm.ge
Neurophage Pharmaceuticals	http://neurophage.com
New Horizons Diagnostics	http://www.nhdiag.com/index.htm
Novolytics	www.novolytics.co.uk
OfmniLytics Inc	www.phage.com
Phage Biotech Ltd	www.phage-biotech.com
Phage International	www.phageinternational.com
Phage Works	No website identified
Phico Therapeutics	www.phicotherapeutics.co.uk
Special Phage Services Pty Ltd	No website identified. Merged into Ampliphi
Targanta Therapeutics	www.targanta.com
Viridax	www.viridax.com

Theses/dissertations searches were conducted on:

- Proquest Dialog's Food and Agriculture section - limited to dissertations and thesis
- DART-Europe
- Ethos (Electronic thesis online service from the British Library)
- Thesis Canada

Results were collated by the Librarian. The Project Manager then selected documents where further details were needed and scanned these to select those for redacting and forwarding to appropriate reviewers. The criteria used for inclusion in the review were as follows:

- Reference to one of the organisms included in the critical review;
- Involve the use of phage as a biocontrol agent in food;
- Published in English.

Table 9: Results from Internet searches

Search	Further details of	Sent to reviewers
Organisation websites and Open Grey	7	0
Company websites	2 (+ articles mentioned confirmed as already identified)	0
Thesis and dissertations	5	(sections only)

3.3 Critical review criteria

All articles and patents from the database searches available from the Campden BRI libraries or from internet sources were redacted, had the protocol scoring sheet attached, and were sent to the two allocated reviewers. Articles and scoring sheets were identified by a pathogen ID code and item number. The scoring sheet assessed the papers for the range of criteria:

- Method of phage propagation available;
- Use of appropriate pathogens/ spoilage organisms for food type used;
- Method of inoculation of food samples available;
- The number of pathogen/spoilage strains used, their source – culture collection or food isolates;
- Method of phage application to the food available;
- Method of phage enumeration available;
- Calculation of phage dose available;
- Number of time points tested;
- Number of samples/replicates used;
- Inclusion of relevant controls;
- Statistical analysis performed on the raw data i.e standard deviations and/or error bars on the graph;
- Analysis of data performed – log reduction calculated;
- Conclusions on the efficacy of the phage/suggested use of the phage;
- Comparison with other published data.

A copy of the scoring sheet is included in Appendix 1 for reference.

The titles and abstracts of those articles and patents not available in-house or only available in non-English texts were screened by the Project Manager, selected ones ordered from document supply sources and sent to reviewers. The review criteria were that each text should include:

- Reference to one of the organisms included in the review;
- Involve the use of phage as a biocontrol agent in food;
- Published in English.

Textbooks with chapters of interest were either borrowed from the British Library or, in one case, ordered for the general library collection and checked by the Project Manager using the same criteria as those stated above. Details of the numbers of references sent for critical review by the expert review panel are on the next page:

Pathogen and ID code	Total number of articles and patents sent to reviewers (20/8/15)
Bacillus (BA)	37
Brochothrix (BR)	2
Campylobacter (CA)	26
Citrobacter (enteric) (CI)	3
Clostridium (CL)	19
Escherichia coli (enteric) (ES)	28
Hafnia (enteric) (HA)	4
Lactic acid bacteria (LA)	33
Listeria monocytogenes (LI)	82
Micrococcus (MI)	0
Pseudomonas (PS)	17
Salmonella (SA)	72
Staphylococcus aureus (ST)	54
STEC (STE)	79

3.4 Critical review results

Protocol scores from the two reviewers in each case were added and the top 25% of items for each pathogen were selected for inclusion in the evidence tables.

Table 10: Numbers of articles for each pathogen

Organism	Number of articles	Sent to reviewers	25% selected for evidence tables
Bacillus cereus	59	38	9
Brochothrix	2	2	1
Campylobacter	36	26	6
Citrobacter	6	3	0
Clostridium botulinum	27	19	5
Escherichia coli	34	28	7
Hafnia	5	4	0
Lactic acid bacteria	55	33	8
Listeria monocytogenes	119	86	21
Micrococcus	1	1	0
Pseudomonas	32	19	4
Salmonella	101	77	19
Staphylococcus aureus	76	56	14
STEC	101	83	21

Details of the scores for all of the references included in the review are listed in Appendix 2 for reference. The selected papers were next forwarded to four expert scientific reviewers for completion of the evidence tables. The reviewers were forwarded an agreed format blank template detailing the key information to be extracted from the papers for both pathogens and spoilage organisms. Each of

the reviewers then populated the table with the data they considered to be most relevant based on the quality of the papers that they were assigned to review.

On completion the evidence tables were forwarded to the project manager for inclusion into the report.

3.5 Overview of information obtained

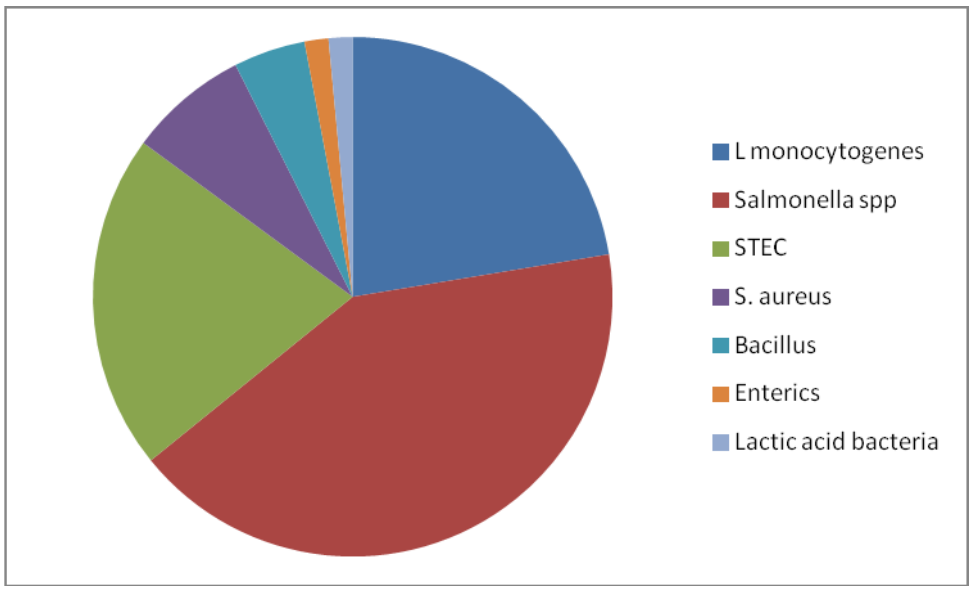
Initial analysis of the papers gathered for the review revealed that 66% of the 621 papers collated documented the use of bacteriophage treatment on pathogens in food and 34% investigated the use of bacteriophage on spoilage organisms. After completion of the critical review 6 organisms (*Brocothrix*, *Campylobacter* spp, *Citrobacter* spp, *Clostridum* spp, *Hafnia* spp and *Micrococcus,*spp) were not taken forward, as the information collated did not meet the criteria devised for this critical review.

All of the papers in the top 25% of score bracket for *Campylobacter* spp. focused on the control of the pathogen in either live chickens or in biofilms. The references that reported on the use of bacteriophage in live animals were excluded as these were outside the scope of the current project. The scope of the review was limited to foods as the authors were informed that previous work on the use of bacteriophage in live animals had already been commissioned.

Analysis of the scores for the spoilage organisms revealed that most of the references sources fell below the threshold score of 40% from the criticial review required for inclusion in the evidence tables. Scores of less than 40% indicate that insufficient detail is available in the text to fully complete the evidence tables within the report.

The percentage distribution of the number of papers for each organism included in the final evidence tables is shown in Figure 1.

Figure 1: Percentage distribution of the number of papers for each organism included in the final evidence tables



It can be seen that by far the highest proportion of the published literature studied the control of key foodborne pathogens (as opposed to spoilage organisms), in food using bacteriophage treatments. An additional observation from the papers collated for STEC was that most of the studies focussed on the

O157 serotype with very little data available for the other 5 serotypes listed in the EU serotypes as highly pathogenic (O26, O103, O111, O145 and O104).

Key finding 7: Scarcity of data available for the use of bacteriophage to control spoilage organisms on foods compared to foodborne pathogens. Research has focussed on key food borne pathogens; *L. monocytogenes*, *Salmonella* spp., STEC and *S. aureus*. *Campylobacter* spp. was not included in the review as the studies on this pathogen investigated its control in biofilms and live animals which were outside the scope of the current review.

Additional points of note were the study designs used to evaluate bacteriophage performance, with specific reference to the number of microbial isolates used, the number bacteriophage applied, as well as the timings of the bacterial spike and bacteriophage treatment. Details of the number of microbial strains and bacteriophage isolates were recorded in the evidence tables and a summary of the information gathered is displayed in Table 11.

Table 11: Summary of the bacteriophage and microbial inoculum used for the published bacteriophage efficacy studies used in the review

Organism	Bacterial spike		Phage treatment	
	Single isolate	Cocktail of isolates	Single isolate	Cocktail of isolates
L. monocytogenes	53%	47%	73%	27%
Salmonella spp	72%	28%	33%	67%
STEC	50%	50%	33%	67%
S. aureus	100%	0%	0%	100%
Bacillus spp	100%	0%	100%	0%
Enterics	100%	0%	100%	0%
Lactic Acid Bacteria	100%	0%	100%	0%

Key finding 8: Many of the studies to date have focused on the use of single isolate spiked samples to determine the performance of the bacteriophage treatment. Some studies have used cocktails of bacteriophage to control the level of microbial contamination in foods.

In addition to the bacteriophage and microbial isolates used, a review of the method of food inoculation used was carried out, with particular emphasis being given to the timings of bacteriophage application. The data revealed that all of the studies used spiked samples where the organism of interest was deliberately introduced into the food sample.

Key finding 9: No information was available on the use of bacteriophage to control natural microbial contamination present on food samples.

One important aspect highlighted in the safety of bacteriophage as biocontrol agents was the timing of application of the treatment to deliver optimum results. Whilst reading the papers for the top 3 pathogens (*L. monocytogenes*, *Salmonella* and STEC), it was noted in most evaluations that the pathogens were always added prior to the addition of the bacteriophage.

Key finding 10: The addition of bacteriophage in most of the studies is carried out following the spike of bacteria followed by a period of 'acclimatisation' on the food matrix.

Furthermore, there was another point raised during the review process that was common across most of the organisms included in the review – key finding 11.

Key finding 11: Direct comparison between the studies is difficult due to the variation in the parameters used, including phage and pathogen dose, mode of inoculation and incubation times and temperatures.

3.5 Evidence Tables

The literature gathered during the search process by the librarians was initially screened as described in section 3.1 and 3.2, to create a list of publications to send out to the review team. These papers were passed to the review team who evaluated each reference using the scoring sheet, and were given a score out of 30. During the scoring process the papers were anonymised to ensure that the assessment process was unbiased by author or literature source. The score sheet was devised to provide a standardised protocol for assessing the quality and reliability of the data collated. The results from the review indicated that there was a wide variability in the scores obtained for some papers. Some of the key reasons for this were that papers:

- Investigated the use of other biocontrol agents other than bacteriophage, such as bacterial endolysins, bacteriocins and antagonistic organisms;
- Reported details of isolation of the bacteriophage only;
- Were written as a review rather than a single published study; or
- Studied bacteriophage that targeted starter culture strains rather than bacteriophage used as biocontrol agents. This was particularly noticeable in the papers for lactic acid bacteria.

The level of detail included in the papers collated, varied depending upon the scope of the study reported. Some studies evaluated commercially produced bacteriophage products on a range of food groups, whereas others explored the potential of bacteriophage isolated from food and the environment. There was a great variability in the format of the results presented in the papers ranging from tables containing calculated log reductions, to graphs with error bars and microbial log reduction values quoted in the text. There were also a few investigations that explored the potential of combination treatments including bacteriophage and other antimicrobial treatments such as high pressure processing (HPP).

The data extracted from the reviewed literature was used to create Evidence Tables for each of the individual organism (Tables 12-18). Data was obtained on as many of the following parameters as possible:

- Food products used and their pH;
- Information on organism studied, details of the isolates used in the research as well as the level of organism spiked into the food and the method of inoculation;
- Name of the bacteriophage/cocktail of bacteriophages used, the level applied to the food and method of inoculation;
- Time and temperature details of the exposure of the bacteriophage to the target organism in the food;
- Level of log reduction achieved of the target organism in the food sample.

An additional screen of papers was also carried out at the evidence table stage to remove the papers containing insufficient data to be relevant to the project. The criteria used were:

- Contained food as the primary matrix;
- Information recorded in at least the following fields; food product, contact exposure conditions, level of phage dose, level of pathogen applied and log reduction achieved per unit;
- A total score (from 2 reviewers) of at least 24 (40%) from the reviewers.

Data has not been included for the following organisms as the references failed to meet the criteria listed above: *Citrobacter* spp; *Hafnia* spp; *Pseudomonas spp*; *Clostridium* spp; *Micrococcus* spp; and *Campylobacter* spp.

Information on the efficacy of the bacteriophage is included in a second table located in Appendix 3. This included:

- Number of bacterial isolates inoculated into the food e.g. single organism or cocktail of organisms;
- Number of bacteriophage strains applied to the food e.g. single bacteriophage or cocktail of bacteriophages;
- Mode of action of the bacteriophage, i.e. whether it is lytic or lysogenic;
- Details of the bacteriophage specificity;
- Whether or not residual activity of the bacteriophage was observed; and
- Any limitations quoted in the study.

Table 12: Evidence table for the biocontrol of *Salmonella* in foods

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Chicken skin	cocktail of x5 φSE phages	30 mins	ambient	N/A	10^9 pfu/ml	4cm^2 sections inoculated with 10^5cfu/cm^2 spread and dried for 30 mins @ 25^{0}C	sections dipped into 100 ml of phage cocktail	1.0 log cfu/cm^2	SA88	90
Energy drink	P22	24, 48 hours	4^{0}C	N/A	10^{12} pfu/ml MOI of 10^8	Inoculated with overnight culture to give 10^4 cfu/ml. incubated at 4^{0}C for 1 hour prior to treatment	1 ml added to 100ml food.	2.1, 2.1	SA22	90
Apple Juice								2.5, 2.1.		
Whole milk								1.7, 4.5		
Skimmed Milk								1.9, 4.3		
liquid whole egg								0.8, 1.0		
Sliced chicken breast	P22	24, 48 hours	4^{0}C	N/A	10^{12} pfu/ml MOI of 10^7	6 x 30g inoculated with 5ml at 10^5 cfu/ml. Incubated at 4^{0}C for 1 hour prior to treatment	5ml inoculated onto the surface of 3 samples using spray dispenser	1.73, 1.83	SA22	90

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Chicken Mince	P22	24, 48 hours	4^0C	N/A	10^{12} pfu/ml	300g inoculated homogeneously with 3 ml at 10^6cfu/ml. Incubated at 4^0C for 1 hour prior to treatment	8ml homogeously included into 150g then 6 balls (25g) produced	1.4, 2.0	SA22	90
Liquid whole egg	P22	24, 48, 72 hours	4^0C	N/A	10^{12} pfu/ml MOI of 10^5	Contaminated with *S.*Typhimurium + x1 undefined isolate I to reach 10^4 cfu/ml. Incubated at 4^0C for 1 hour prior to treatment	added to 100 ml food	1.2, 1.5, 2.0	SA22	90

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Liquid whole egg	P22	24, 48 hours	4^0C	N/A	10^{12} pfu/ml MOI of 10^5	Contaminated with *S.*Typhimurium + x3 undefined isolates to reach 10^4 cfu/ml. Incubated at 4^0C for 1 hour prior to treatment	added to 100 ml food	1.3-2.0 (24hr) 1.4-2.0 (48hr)	SA22	90
Mung Bean (250g)	Cocktail of 6 phages	20 mins then stored and sprouted for 4 days	ambient	N/A	10^6 pfu/ml	soaked in a cocktail (250ml) of 11 overnight *Salmonella* strains	Soaking in 250 ml suspension dried overnight at ambient.	3.4	SA224	90
	Cocktail of 6 phages + *E. asburiae* (10^6 cfu/ml)							5.9		
Alfalfa seeds (100g)	Cocktail of 6 phages + *E. asburiae* (10^6 cfu/ml)							6.8 log	SA224	90

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Pig Skin	Cocktail of 4 phages; PC1	1, 48, 96 hr	4^0C	N/A	10^4 – 10^7 to give MOI 0.01 to 1000	4 cm^2 pieces inoculated at a level of 10^3 - 10^6 cfu/cm^2. spread and dried for 1 hr.	4 cm^2 pieces inoculated, spread, dried for 1 hr at ambient	MOI<10 -0.2 – 0.4 MOI10 0-1, 0.4-3.3, 1-3.5 MOI 100 0.9, 0.5, 3.5 MOI 1000 1.2, 1.4, 1.3 MOI 10,000 0.4, 0.5, 1.5	SA182	83
Soybean milk	Single ST1	3, 6, 9, 24 hr	37^0C	6.7	10^6 – 10^8 to give MOI of 1 to 100	30ml inoculated to a level of 10^6 cfu/ml	30ml of sample inoculated	MOI 1 0 MOI 10 0.5[a], 1.2[a], 1.7[a], <LOD MOI 100 <LOD by 3 hrs	SA39	82
Chicken skin	wksl3	1, 7 days	8^0C	N/A	10^8 to give a MOI of 5 x10^3	4cm^2 skin pieces inoculated by spraying	4cm^2 skin pieces inoculated by spraying to give 10^3 – 10^4 cfu/cm^2 dried under blowing air for 1 hr at 8^0C	3.0, 2.4	SA97	82

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Chicken skin	AV-08	1 day	4^0C	N/A	10^{13} to give MOI of 10,000	$4cm^2$ sections inoculated by pipetting 1 ml of 10^9 cfu/ml culture store for 1 hour.	Application by pippetting 1ml on skin surface store for 1 hour at 25^0C.	5.2	SA129	82
Hot dogs	FO1-E2	2 days	8 or 15^0C	N/A	3x 10^8 in sample	Diluted overnight culture inoculated into product to give a level of 10^3cfu/g. stored at 8 or 15^0C for 1-2 hrs.	Added to give concentration of 3 x10^8 pfu/g	3.0	SA150	82
Cooked sliced turkey breast								3[a]		
Mixed seafood								1.9		
Chocolate milk								3[a]		
Egg yolk								2[a]		
Dried pet food (kibble)	SalmoLyse	1 hr	ambient	N/A	10^7, 10^8, 10^9	Diluted overnight culture at 10^6 cfu/g was added to 150g product. 5 x 0.9ml was added with mixing between each addition. Stored for 1 hr at ambient.	Surface spray to a final level of 10^5, 10^6, 10^7 pfu/g on product. Stored at ambient for 1 hr.	10^5 pfu/g 0.75 log pfu reduction	SA12	77
								10^6 pfu/g 1.37 log pfu reduction		
								10^7 pfu/g 2.05 log pfu reduction		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Tomatoes	Cocktail of 6 phages + *E. asburiae* (10^3 cfu/ml)	7 days	15^0C	N/A	10^3 pfu/ml MOI = 0.001	Fruits placed in a suspension of 10^6 cfu/ml *Salmonella* for 10 min then treated	Fruit placed in a suspension of phage cocktail + *E. asburiae* for 10 min. Subjected to 3 vacuum release cycles	0.1 to -0.1 for surface and internal	SA12	77
	Cocktail of 6 phages + *E. asburiae* (10^6cfu/ml)	7 days	15^0C	N/A	10^6 pfu/ml MOI = 1			0 to -0.1 for surface and internal	SA12	77
Broccoli	SalmoFresh	24 hours	10^0C	N/A	10^8 pfu/g	10g produce spot inoculated with 20µl in 10 random spots	sprayed to deliver 100 - 200µl. Stored at ambient for 10 min.	2.79	SA73	63
Cantaloupe								1.78		
Strawberries								0.49		
Broccoli	SalmoFresh + Produce wash Fit L (levulinic acid, grapefruit oil, terpene) 1:88 conc	1, 5 mins	10^0C	N/A	10^8 pfu/g	10g produce spot inoculated with 20µl in 10 random spots	sprayed to deliver 100 - 200µl. Stored at ambient for 10 min.	3.83, 4.55	SA73	63
Cantaloupe								3.69, 3.96		
Strawberries								4.63, 4.63 (to LOD)		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Pig skin	Cocktail 3 phages	3, 6 hours	33^0C	N/A	10^{10} pfu/ml MOI = $4.4x10^4$	1.8 ml of 10^6 cfu/ml inoculated and spread onto 900cm^2 pieces. Divided into 2 Dried at ambient for 30 mins	Surface of skin sprayed with 4 ml, dried for 30 mins at ambient.	>2.2 – 2.9, 2 - >4	SA84	63
Chicken		1, 2, 5, 7 days	4^0C		10^9 pfu/ml MOI = $1x10^3$	30g pieces immersed in 60ml 10^6 cfu/ml suspension for 5 mins at ambient. Dried at ambient for 15 mins	In bag, immersed in 100ml for 5 mins with agitation at room temperature	0.7 – 1.6, 1 – 1.6, 1.4, 0.9 – 2.2		
Whole eggs		2 hr	Ambient		10^{10} pfu/ml MOI = $1x10^3$	Dipping in 200ml of 10^7 cfu/ml, suspension for 5 mins at ambient. Dried for 3 hours at ambient	Sprayed.	0.9		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Romaine lettuce		30, 60 mins	Ambient		10^9 pfu/ml MOI = $1x10^4$	Immersed in l 10^5 cfu/ml suspension (1:10 w/v) for 5 mins at ambient. Dried for 15 mins at ambient.	In bag, 25 g lettuce immersed in 100ml for 60 mins with agitation (100rpm)	1.9 – 3.4, 2.2 – 3.9		
Mixed salad leaves	Cocktail 3 phages	1, 2, 3, 4, 5, 6, 7 days	4^0C	N/A	10^8 pfu/g MOI = $1x10^4$	80g samples were inoculated to achieve 10^3cfu/g. Incubated at 4^0C for 2 hours.	Added to food and mixed for 2 mins.	≈ 2log (1day)	SA 281	14, 20
Beansprouts								≈ 2log (1day),		
Cooked chicken breast								≈ 2log (1day)		
Raw skinless chicken breast								1.75 (1 day)		

Table 13: Evidence table for the biocontrol of *Listeria monocytogenes* in foods

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
RTE oven roast turkey breast aerobic storage	Cocktail 2 phages	6d	4°C	N/A	10^9 per membrane	0.1ml of 1x 10^3 cfu per ml spotted on 25 g meat.	Phage immobilised on membrane transferred to meat surface	>2	LI73	83
		9d	4°C					0.5		
		12d	4°C					0.6		
		4d	10°C					<1		
		6h	25°C					<1		
		24h	25°C					1.4		
RTE oven roast turkey breast MAP storage		24h	4°C					>2		
		9d	4°C					2.2		
		12d	4°C					1.48		
		15d	4°C					1.7		
		24h	10°C					>2		
		6h	25°C					<1,		
		24h	25°C					1.37		
RTE oven roast turkey breast vacuum storage		24h	4°C					>2		
		24h	10°C					>2		
		6h	25°C					1.0, 1.63		
		24h	25°C					1.63		
Soft cheese Minas Frescal	Listex P100	30min	21°C	N/A	8.3×10^7	1ml of 1 x 10^5 per 30g cheese	1ml inoculation onto cheese	2.3	LI11	77
Soft cheese Coalho		30min	21°C					2.1		
Soft cheese Minas Frescal		7d	10°C					1.0		
Soft cheese Coalho		7d	10°C					0.8		
Melon	Listshield cocktail	48h	10°C	5.92	1.5×10^6 per slice fruit, 5×10^8 per ml in juice	1.0×10^5 per ml spot inoculated	15 µl spot inoculation of 1.0×10^8 per ml	-0.5-0.0	LI21	77
		120h						-3.0 to- 1.5		
		192h						-3.8 to -3.2		
Pear	Listshield cocktail	48h	10°C	4.91				-0.5 – 0.5		
		120h						-2.0 to -1.5		
		192h						-2.8 to -2.0		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Apple	Listshield cocktail	48h	10°C	3.76				-0.3 – 0.5		
		120h						-1.9 -0		
		192h						-2.0 to -0.5		
Melon juice	Listshield cocktail	48h	10°C	5.77				1.0		
		120h						3.1-3.5		
		192h						4.2-4.7		
Pear juice	Listshield cocktail	48h	10°C	4.61				2.4-3.1		
		120h						1.7-3.5		
		192h						1.9-2.4		
Apple juice	Listshield cocktail	48h	10°C	3.70				-0.1-.0.5		
		120h						1.4-2.1		
		192h						1.4-2.9		
Lettuce – long leaf green	Listshield cocktail	5min	ambient	N/A	1.0 x10^7 - 1.0 x10^8	Surface inoculum 2 x10^3 cfu per g	No detail given	0.5-1.1	LI119	77
Apple - Gala	Listshield cocktail	24h	4°C	N/A	1.1 x10^6	Surface inoculum 1 x10^4 per g	0.1ml per 100g apple	1.0		
		48h						1.0		
		72h						0.7		
Hard pasteurised cheese	Listshield cocktail	5min	4°C	N/A	1.0 x10^8	Surface inoculum 1 x10^4 cfu per g	1ml per 100g	0.7		
		24h						0.7		
		48h						0.5		
Smoked salmon	Listshield cocktail	24h	4°C	N/A	9 x 10^5	Surface inoculum Of 1 x10^3 cfu per g	0.2ml per 100g	0.4		
		24h			2 x 106			1.0		
Frozen entrees	Listshield cocktail	24h	frozen	N/A	1.0 x10^7 per g	Surface inoculum of 5 x 10^3 cfu per g	2ml treatment	3.0 (stat significant)		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Vacuum packed Cooked turkey	Listex P100	30min	4, 10°	N/A	2.0 x 10^7 per cm^2	Surface inoculation of 10^3 per cm^2	Surface treatment of 0.1ml	1.4, 1.4	LI30	75
		1d						0.9-1.8		
		7d						1.5-1.6		
	Listex P100+ 2.8% potassium lactate	30min	4, 10°C					1.3		
		1d						2.0		
		7d						1.3-2.1		
Vacuum packed Roast beef	Listex P100	30min	4, 10°C					1.1		
		1d						1.5-1.8		
		7d						0.0-0.9		
	Listex P100+ 2.8% potassium lactate + 0.2% sodium diacetate	30min	4, 10°C					1.0		
		1d						1.1-1.5		
		7d						1.5-1.6		
Queso fresco cheese	Listex P100	1h	4°C	N/A	2.5 x 10^7	Surface inoculation of 10^4 cfu per g	Total Surface treatment of 1.25ml of which 0.25 is phage suspension	1.2-2.3	LI58	75
		4h						2.2-4.0		
		24h,						3.4-5.6		
		7d						3.1-3.3		
		14d						2.0-2.2		
	Listex P100 + 200 ppm/g lauric arginate (LAE)	1d,						2.9-3.4		
		7d						1.6-2.2		
		14d						0.7-1.4		
	Listex P100 + 2.8% potassium lactate and 0.2% sodium diacetate (PL-SD)	1d,						3.6		
		7d						2.8-3.2		
		14d						2.4-2.8		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Fresh channel catfish fillets	Listex P100	30min	10°C	N/A	10^8	Spot inoculation to give 10^4 cfu per g	0.1ml surface treatment	1.4-2.1	LI84	75
		4d						0.2-1.2		
		10d						0.2-0.5		
Hot dog sausages	A511	1d	6°C	N/A	3.0 x 10^8	10^3 cfu per g	0.5-1.0ml surface treatment	2.2-3.0	LI96	75
		3d						1.7-3.0		
		6d						1.5-3.2		
		13d						0.5-3.2		
Cooked sliced turkey meat		1d						1.8-2.2		
		3d						0.2-1.0		
		6d						-0.5-2.0		
Smoked salmon		1d						0.0-1.1		
		3d						0.2-1.4		
		6d						0.0-0.8		
Mixed seafood		1d						1.5-1.7		
		3d						0.9-1.7		
		6d						0.0		
Chocolate milk		1d						2.2-3.0		
		3d						3.0		
		6d						3.0		
		13d						3.0		
Mozzarella cheese brine		1d						3.0		
		3d						3.0		
		6d						3.0		
		13d						3.0		
Iceberg Lettuce		1d						2.0-2.2		
		3d						1.3-2.8		
		6d						1.5-2.0		
Cabbage		1d						2.2-3.0		
		3d						2.2-3.0		
		6d						2.2-3.0		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Hot dog sausages	Listex P100	1d	6°C	N/A	3.0×10^8	10^3 cfu per g	0.5-1.0ml surface treatment	2.0-2.8		
		3d						2.8		
		6d						2.2-2.8		
Smoked salmon		1d						1.7-2.8		
		3d						1.4		
		6d						0.6-1.4		
Mixed seafood		1d						2.0-3.0		
		3d						1.5		
		6d						1.0		
Cabbage		1d						1.8-2.0		
		3d						1.5		
		6d						1.0		
Brazilian fresh sausage	Listex P100	20min	4°C	N/A	10^8	1ml of 10^5 per ml spot inoculation	1.0ml surface treatment	2.5-4.0	LI74	72
		10d						2.5-3.7		
Raw salmon fillet	Listex P100	30min	ambient	n/a	10^8 pfu per g	10^4 per g	0.1ml surface treatment of flesh side	2.7-3.2	LI90	63
			4°C					2.7-3.5		
		2h	ambient					3.0-3.2		
			4°C					2.9-3.1		
		30min	ambient			10^3 per g		2.6-3.0		
			4°C					2.5-2.7		
		2h	ambient					2.8-3.0		
			4					2.4-2.6		
		30min	ambient			10^2 per g		1.7		
			4°C					1.7		
		2h	ambient					1.8		
			4°C					1.6		
RTE chicken breast roll vacuum packed	Single phage isolates from sheep faeces	192	5°C	N/A	2.5×10^6 per cm^2	10^4 per cm^2	20µl surface treatment	1.0-2.5	LI75	63
		288						1.0		
		432						1.0		
		192				10^2 per cm^2		1.8		
		288						2		
		432						2		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Beef bologna	Cocktail 6 phages Single phage FWLLm3	24h, 72h, 168h 10d	4°C	N/A	1x 10^9 per ml	2.0 x 10^3 per cm^2	1ml per 500cm^2	2.34, 2.69, 2.45	LI106	63
Beef frankfurters								1.91, 1.25, 1.45		
Roast beef								1.62, 1.79, 1.35		
Roast turkey								1.49, 1.36, 1.33		
Sliced ham								2.07,2.16, 1.16		
Smoked turkey								1.48, 1.88, 1.83		
Turkey bologna								2.47, 2.57, 2.75		
Turkey frankfurters								1.71, 1.18, 1.28		
Turkey pastrami								1.48, 1.88, 1.83		
Turkey salami								1.99, 1.97, 1.90		
Lebanon bologna								0.62, 1.00, 1.00		
Extended shelf life (ESL) milk	Single phage FWLLm1	2d	4°C	n/a	10^6	10^4 cfu per ml	Phage to bacteria ratio of 10:1 for LWLLm1 and 100:1 for FWLLm3. Bacteriocins added at a concentration of 584 AU/ml	4.0	LI1	55
	Single phage FWLLm3 plus bacteriocin	4d			10^5			6.0		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Cooked ham	Listex P100	3d	10°C	n/a	5 x 10^7 per cm^2	10^4 per g = 10^3 per cm^2	0.1ml spread on meat surface with glass rod	0.5	LI98	50
		7d						-1.5		
		14d						-3.5		
		28d						-4		
	Listex P100 plus 10^3 antagonisitic L. sakeii	3d	10°C					1.0		
		7d						0		
		14d						0		
		28d						0		
		3d	4°C					1		
		7d						1		
		14d						0.8		
		28d						1		
Cold smoked salmon	Listex P100	24h	4	n/a	10^8 per g	10^4 per cm^2	0.1ml added to each sample. During vacuum packing the suspension was spread evenly on fish surface	2.5-3.0	LI14	40
	Listex P100 plus 200ppm LAE	24h	4					1.2-1.7		
	Listex P100 plus 50ppm nisin	24h	4					3.5		

Table 14: Evidence table for the biocontrol of Shiga toxin producing *E. coli* (STEC) in foods

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Green leaf lettuce (Modified atmosphere packaged)	EcoShield cocktail	15 days	4 or 10ºC	-	2.5E+8 PFU /cm2 to	Added to food at a level of 5.3 log cfu/cm^2 and air-dried (host attachment)	Sprayed to give 8.4 log pfu/cm^2 on food after host attachment	2.9 log CFU /cm2	STE349	45
Spinach (Modified atmosphere packaged)	EcoShield cocktail	15 days	4 or 10ºC	-	2.5E+8 PFU /cm2	Added to food at a level of 5.3 log cfu/cm^2 and air-dried (host attachment)	Sprayed to give 8.4 log pfu/cm^2 on food after host attachment	2.2 log CFU /cm2	STE349	45
Romaine lettuce (Modified atmosphere packaged)	EcoShield cocktail	15 days	4 or 10º C	-	2.5E+8 PFU /cm2	Added to food at a level of 5.3 log cfu/cm^2 and air-dried (host attachment)	Sprayed to give 8.4 log pfu/cm^2 on food after host attachment	2.8 log CFU /cm2	STE349	45
Chicken skin	AV-08	1, 5 days	4ºC	-	1E+12 PFU /ml MOI 10^4	1 ml of culture added to chicken skin to give log 8 cfu/cm^2 and attached for 1h	After 1 hour attachment 1 ml of 10^{13} pfu/ml applied by pippette	3.5, 4.4, log cfu/4cm^2	STE149	45
Cabbage	ECP4	3 hours	37ºC	-	1E+8 PFU /ml	Added to food to give final concentration of 4 log cfu/g	Added to food to give final concentration of 8 log pfu/g	4 log	STE15	60
Vegetable juice	ECP4	5 hours	37ºC	-	1E+8 PFU /ml	Added to food to give final concentration of 4 log cfu/g	Added to food to give final concentration of 8 log pfu/g	Up to1 log CFU /ml	STE15	60

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Fermented milk	Cocktail (DT1 & DT6)	1 h	37ºC	7.2 (start) - 4.0 (end of fermentation)	1E+7 – 1E+8 /ml to give MOI 10^4	Added to reconstituted skim milk, with S. thermophilus culture at 1%(v/v) to give final concentration of $5x10^2$ – $5x\ 10^3$ cfu/ml	Added to reconstituted skim milk, with *S. thermophilus* culture	MOI log 4 = 3 log cfu/ml (0157 STEC)	STE18	68
Fermented milk	DT1	1, 8, 24 h	37º C	7.2 (start) - 4.0 (end of fermentation)	1E+7 – 1E+8 /ml to give MOI 10^4 - 10^5	Added to reconstituted skim milk, with *S. thermophilus* culture at 1%(v/v) to give final concentration of $5x10^2$ – $5x\ 10^3$ cfu/ml	Added to reconstituted skim milk, with *S. thermophilus* culture	MOI log 5 3 log cfu/ml (DH5α 1h) MOI log 5 3 log cfu/ml (0157 STEC 8 h) MOI log 4 2log reduction (EPEC920 1 hour) subsequent growth over 24 hr	STE18	68
Fermented milk	DT6	8, 24 h	37ºC	7.2 (start) - 4.0 (end of fermentation)	1E+7 – 1E+8 /ml to give MOI 10^3 - 10^5	Added to reconstituted skim milk, with *S. thermophilus* culture at 1%(v/v) to give final concentration of $5x10^2$ – $5x\ 10^3$ cfu/ml	Added to reconstituted skim milk, with *S. thermophilus* culture	MOI log 5 =3 log cfu/ml (O157 STEC 8 h) MOI log 3 <1 log (nonO157 STEC 24hr)	STE18	68

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Raw beef	FAHEc1	24 h	5ºC	-	log 6.9 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 3.6 cm^2 attached for 10 min	20µl added to meat surface, following attachment	2.4 log CFU /cm2	STE68	73
					log 4.1 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 1.0/ cm^2 attached for 10 min		0.5 log CFU /cm2		
Raw beef	FAHEc1	24 h	24º C	-	log 3.7 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 3.7/cm^2 attached for 10 min	20µl added to meat surface, following attachment	-0.6 log CFU /cm2	STE68	73
					log 6.9 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 3.3/ cm^2 attached for 10 min		-0.2 log CFU /cm2		
					log 5.2 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 1.3/ cm^2 attached for 10 min		-2.2log CFU /cm2		
					log 1.2 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 0.6/ cm^2 attached for 10 min		-3.7 log CFU /cm2		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Cooked beef	FAHEc1	24 h	5ºC	-	log 8.0 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 4.4 cm^2 attached for 10 min	20µl added to meat surface, following attachment	4.3 log CFU /cm2	STE68	73
					log 5.9 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 1.3 cm^2 attached for 10 min		1.1 log CFU /cm2		
			24º C		log 5.1 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 4.6/cm^2 attached for 10 min	20µl added to meat surface, following attachment	-0.9 log CFU /cm2	STE68	73
					log 7.9 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 5.8/ cm^2 attached for 10 min		1.7 log CFU /cm2		
					log 5.5 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 0.7/ cm^2 attached for 10 min		>0.7log CFU /cm2 to LOD		
					log 1.0 pfu/cm^2 on meat	20µl added to 2x2 cm^2 meat surface, to level of log 1.1/ cm^2 attached for 10 min		-1.5 log CFU /cm2		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Sterilised Milk	Cocktail (BCEP2 & BCEP6)	5 h	37ºC	-	1E+8 PFU /ml	Added to milk to a final concentration of 10^4 cfu/ml	Added to milk to give MOI of 10^4	2 log	STE70	63
Strawberry	EcoShield BC Cocktail	24 h	10ºC	-	1E+8 PFU /g	10 random spots of 20µl applied to surface, air dried for 30-45 min to give levels of 5 – 6 log cfu/g	Sprayed 100-200µl onto surface following air drying	0.53	STE94	70
Cantaloupe								0.74		
Broccoli								0.68		
Sliced raw beef	FAHEc1	1 h	37º C	-	log 8.5 pfu/2cm^2 piece	0.1 ml added to meat surface to give 1.4 x 10^4 cfu/2 cm^2 piece and allowed 1 h @ 37ºC for attachment	0.1 ml added to meat surface after attachment	> 2.6 log CFU / piece (to LOD)	STE102	87
					log 7.5 pfu/2cm^2 piece			1.8 log CFU / piece		
					log 6.5 pfu/2cm^2 piece			1.3 log CFU / piece		
					5.4 pfu/2cm^2 +piece			0.5 log CFU / piece		
Sliced raw beef (carcass cooling simulation)	FAHEc1	14 h	35ºC – 10ºC (cooling curve)	-	log 7.5 pfu/2cm^2 piece	Added to meat surface to give 10^3 cfu/2 cm^2 piece and allowed 1 h for attachment	Added to meat surface after attachment	1.6 log CFU / piece (to LOD)	STE102	87
					log 5.5 pfu/2cm^2 piece			0.8 log CFU / piece		
					log 3.6 pfu/2cm^2 piece			1.0 log CFU / piece		
Sliced raw beef (hot-boned beef cooling simulation)	FAHEc1	18 h	35ºC – 5ºC (cooling curve)	-	log 5.7 PFU / piece	Added to meat surface to give 10^3 cfu/2 cm^2 piece and allowed 1 h for attachment	Added to meat surface after attachment	Up to 1.0 log CFU / piece	STE102	87
					log 6.9 PFU / piece			1.4 log CFU/piece		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Baby spinach	BEC8 cocktail	10 min, 1h & 24 h	4ºC	-	1E+7 PFU /ml to give MOI of 1,	Added 20µl spot to leaf surface of high (10^6) cfu/ml inoculum , air dried 1 h	Added 100 µl spot to leaf surface on top of dried host spot	0.26, 0.16,0.40 log CFU / leaf	STE218	77
			8ºC					0.39, 0.68, 1.21 log CFU / leaf		
			23ºC					1.0, 1.28, 1.32 log CFU / leaf		
			37ºC					0.81, 1.94, 2.86 log CFU / leaf		
			4ºC		1E+7 PFU /ml to give MOI of 10	Added 20µl spot to leaf surface of medium (10^5) cfu/ml inoculum , air dried 1 h		0.56, 0.98, 1.66 log CFU / leaf		
			8ºC					0.83, 1.3, 2.45 log CFU / leaf		
			23ºC					0.75, 2.3, 3.0 log CFU / leaf		
			37ºC					1.64, 2.47, 2.53 log CFU / leaf		
			4ºC		1E+7 PFU /ml to give MOI of 100	Added 20µl spot to leaf surface of low (10^4) cfu/ml inoculum , air dried 1 h		0.41, 1.79, 1.5 log CFU / leaf		
			8ºC					1.37, 2.18, 2.81 log CFU / leaf		
			23ºC					2.01, 2.43, 3.53 log CFU / leaf		
			37ºC					1.73, 3.12, 3.08log CFU / leaf		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Baby romaine lettuce	BEC8 cocktail	10 min, 1h & 24 h	4ºC	-	1E+7 PFU /ml to give MOI of 1,	Added 20µl spot to leaf surface of high (10^6) cfu/ml inoculum , air dried 1 h	Added 100 µl to leaf surface on top of dried host spot	0.22, 0.51, 0.67 log CFU / leaf	STE218	77
			8ºC					0.37, 0.81, 0.87 log CFU / leaf		
			23ºC					0.97, 0.59, 1.68 log CFU / leaf		
			37ºC					0.22, 0.94, 3.68 log CFU / leaf		
			4ºC		1E+7 PFU /ml to give MOI of 10	Added 20µl spot to leaf surface of medium (10^5) cfu/ml inoculum , air dried 1 h		0.99, 1.32, 1.83 log CFU / leaf		
			8ºC					0.77, 1.16, 1.51 log CFU / leaf		
			23ºC					1.4, 1.42, 2.05 log CFU / leaf		
			37ºC					1.66, 2.1, 3.21 log CFU / leaf		
			4ºC		1E+7 PFU /ml to give MOI of 100	Added 20µl spot to leaf surface of low (10^4) cfu/ml inoculum , air dried 1 h		1.51, 1.77, 2.02 log CFU / leaf		
			8ºC					1.81, 1.92, 1.94 log CFU / leaf		
			23ºC					1.99, 2.17, 3.78 log CFU / leaf		
			37ºC					2.77, 3.49, 3.57 log CFU / leaf		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Spinach on Harvester Blades	Cocktail of 6 strains	2 h	22 °C	-	1E+8 PFU /ml to give MOI log 3	Blades incubated in spinach extract inoculated with E. coli O157 for 48 hrs, rinsed in sterile water and blade, air dry 30 min to give initial level of 4.87 log cfu/blade	Airbrush spray after air drying. 100µl/blade of log 8 pfu/ml phage cocktail	4 log CFU /blade	STE221	60
Fresh cut cantaloupe	Cocktail (ECP-100)	0, 2, 5, & 7 days	4ºC	-	8.3 log pfu/ml	25µl of 6.15 log cfu/ml added to 0.5cm diameter well made in 25mm wedge	25µl of phage added to 0.5cm diameter well made in 25mm wedge to give 3.74 log cfu/g	0.21, 2.97, 2.46, 2.78 log CFU /ml	STE328	75
Fresh cut iceberg lettuce	Cocktail (ECP-100)	0, 1 & 2 days	4ºC	-	5.98 log pfu/cm^2	50µl (8-10 drops) of 4.7 log cfu/ml on leaf, air dry for 1 hour at 25ºC	Airbrush spray after air drying to give 2.64 log cfu/g	1.92, 2.42, 2.06 log CFU /ml		
Beef	Cocktail (T5, T1, T4, O1)	3, 24, 144h	4ºC	-	10^8 PFU /cm2 (MOI 1000)	0.1 ml of 10^5cfu/ml added to ≈ 11g meat (to give≈10^3 cfu/g), air dry 10 min	0.1 ml of each level added to meat after air drying	0.4, 0.6, 1.4 log CFU /cm^2	STE344	45
					10^6 PFU /cm2 (MOI 10)			0.3, 0.4, 0.4 log CFU /cm^2		
Beef	Cocktail (T5, T1, T4, O1)	3, 6h	22ºC	-	10^8 PFU /cm2(MOI 1000)	0.1 ml of 10^5cfu/ml added to ≈ 11g meat (to give≈10^3 cfu/g), air dry 10 min	0.1 ml of each level added to meat after air drying	1, 1.2 log CFU /cm^2		
					10^6 PFU /cm2 (MOI 10)			0.7, 0.9 log CFU /cm^2		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Beef	Cocktail (T5, T1, T4, O1)	144 h	37º C	-	10^8 PFU /cm2 (MOI 1000)	0.1 ml of 10^5cfu/ml added to ≈ 11g meat (to give≈10^3 cfu/g), air dry 10 min	0.1 ml of each level added to meat after air drying	0.8 log CFU /cm^2		
					10^6 PFU /cm2 (MOI 10)			0.9 log CFU /cm^2		
Raw beef	Cocktail (EcoM-AG2, EcoM-AG3, EcoM-AG10) Immobilised.	6 & 12days	4ºC	-	4.5E+9 PFU / membrane	0.1 ml of 10^3 cfu/ml added to meat (25g), to give 2.2 log cfu/g, left to attach for 10 min at ambient	Add to meat after attachment, as a membrane in direct contact with the meat	1, >1 log CFU /g (below LOD)	STE208	62
		4 days	10ºC					<1 log CFU /g		
		48 h	25ºC					No effect	STE348 (same experiment as STE 208)	83
Raw beef	Cocktail (EcoM-AG2, EcoM-AG3, EcoM-AG10)	1 day	4ºC	-	1x 10^9 pfu/ml	0.1 ml of 10^3 cfu/ml added to meat (25g), to give 2.2 log cfu/g, left to attach for 10 min at ambient	0.1 ml of phage cocktail added to meat (25g) to produce an MOI of log 5	1.2 log cfu/g	STE347	82

Table 15: Evidence table for the biocontrol of *Staphylococcus aureus* in foods

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Cheese	Cocktail 1 (Team1, P68, LH1-MUT)	14 days	4 °C	6.4 (at start)	1.50E+07 1.50E+08 per ml	Added to whole pasteurised ultra-filtered milk, supplemented with 0.4 g/L $CaCl_2$ at 10^6 per ml	Added to whole pasteurised ultra-filtered milk, supplemented with 0.4 g/L $CaCl_2$	>4 log CFU /ml >4 log CFU /ml	ST76	78
Cheese	Cocktail 2 (Phi812, 44AHJD, Phi2)	14 days	4° C	6.4 (at start)	1.50E+07 1.50E+08 per ml	Added to whole pasteurised ultra-filtered milk, supplemented with 0.4 g/L $CaCl_2$ at 10^6 per ml	Added to whole pasteurised ultra-filtered milk, supplemented with 0.4 g/L $CaCl_2$	>4 log CFU /ml >4 log CFU /ml	ST76	78
Cheese (Fresh)	Cocktail (IPLA35 & IPLA88)	6h 14 days	25 °C (manufacture) 4 C (storage)	6.6 (manufacture), 4.2 (storage)	1E+6 /ml	Added to whole pasteurised milk, at 10^6 per ml	Added to whole pasteurised milk, supplemented with 0.02% $CaCl_2$ and starter culture	>4 log CFU /g >4 log CFU /g	ST33	75
Cheese (Hard)	Cocktail (IPLA35 & IPLA88)	24h 30 days	32 °C (manufacture) 11 C (storage)	6.01 (manufacture), 4.9 (storage)	5E+6 /ml	Added to whole pasteurised milk, at 10^6 per ml	Added to whole pasteurised milk, supplemented with 0.02% $CaCl_2$ and starter culture	2.1 log CFU /g >5 log CFU /g	ST33	75
Pasteuried whole fat milk	Cocktail (IPLA35 & IPLA88)	4 h 24h 48h	25 °C	-	2E+8 /ml	Added to whole pasteurised milk at 10^4 cfu per ml	Added to whole pasteurised milk	0.2-3.0 log cfu per ml 4 log cfu per ml 4 log cfu per ml	ST35	73

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
Pasteurised whole fat milk	Cocktail (IPLA35 & IPLA88)	4h	25°C	-	2E+8 /ml	Added to whole pasteurised milk at 10^6 cfu per ml	Added to whole pasteurised milk	3 log cfu per ml	ST35	73
		24h						3 log cfu per ml		
		48h						-0.5 log cfu per ml		
Pasteurised whole fat milk	Cocktail (IPLA35 & IPLA88) with HPP treatment of 400mPA	4h	25°C	-	2E+8 /ml	Added to whole pasteurised milk at 10^4 cfu per ml	Added to whole pasteurised milk	4 log cfu per ml	ST35	73
		24h						4 log cfu per ml		
		48h						3.4 log cfu per ml		
Pasteurised whole fat milk	Cocktail (IPLA35 & IPLA88) with HPP treatment of 400mPA	4h	25°C	-	2E+8 /ml	Added to whole pasteurised milk at 10^6 cfu per ml	Added to whole pasteurised milk	2.5-4.2 log cfu per ml	ST35	73
		24h						2.8-4.0 log cfu per ml		
		48h						6 log cfu per ml		
Pasteurised whole fat milk	Cocktail (IPLA35 & IPLA88)	2h	37 °C	-	1E+6-1E+8 /ml	Added to whole pasteurised milk at 10^6 cfu per ml	Added to whole pasteurised milk	6.0 log cfu per ml	ST51	93
		4h						6.0 log cfu per ml		
		8h						6.0 log cfu per ml		
		2h			1E+6-1E+8 /ml	Added to whole pasteurised milk at 10^4 cfu per ml		-1.0 log cfu per ml		
		4h						4.0 log cfu per ml		
		8h						4.0 log cfu per ml		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
		2h			1E+2-1E+3 /ml	Added to whole pasteurised milk at 10^6 cfu per ml		-1.5 log cfu per ml		
		4h						6.0 log cfu per ml		
		8h						6.0 log cfu per ml		
		2h			1E+2-1E+3 /ml	Added to whole pasteurised milk at 10^4 cfu per ml		-1.0 log cfu per ml		
		4h						-3.0 log cfu per ml		
		8h						-0.5 log cfu per ml		
Pasteurised whole fat milk	Cocktail (IPLA35 & IPLA88)	2h	15 °C	-	1E+6-1E+8 /ml	Added to whole pasteurised milk at 10^6 cfu per ml	Added to whole pasteurised milk	6.0 log cfu per ml	ST51	93
		4h						6.0 log cfu per ml		
		8h						6.0 log cfu per ml		
		2h			1E+6-1E+8 /ml	Added to whole pasteurised milk at 10^4 cfu per ml		0.5 log cfu per ml		
		4h						1.7 log cfu per ml		
		8h						4.0 log cfu per ml		
		2h			1E+2-1E+3 /ml	Added to whole pasteurised milk at 10^6 cfu per ml		0 log cfu per ml		
		4h						0 log cfu per ml		
		8h						0 log cfu per ml		
		2h			1E+2-1E+3 /ml	Added to whole pasteurised milk at 10^4 cfu per ml		0 log cfu per ml		
		4h						0 log cfu per ml		
		8h						0 log cfu per ml		

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Pathogen	Phage		ID	% Review score
UHT whole fat milk	φA72	2h	37 °C	-	1E+4-1E+5 /ml	Added to UHT whole fat milk	Added to UHT whole fat milk	-1.3 to -1.5 log cfu per ml	ST67	77
		4h						-1.6 log cfu per ml		
		8h						-1.7 log cfu per ml		
UHT whole fat milk	φH5	2h	37 °C	-	1E+4-1E+5 /ml	Added to UHT whole fat milk	Added to UHT whole fat milk	0 log cfu per ml	ST67	77
		4h						-1.2 log cfu per ml		
		8h						log cfu per ml -0.8		
UHT whole fat milk	Cocktail (φA72 & φH5)	2h	37 °C	-	1E+4-1E+5 /ml	Added to UHT whole fat milk	Added to UHT whole fat milk	0 log CFU /ml	ST67	77
		4h						-0.5 cfu per ml		
		8h						-0.3 cfu per ml		
Pasteurised whole fat milk	Cocktail (φA72 & φH5)	2h	37 °C	-	1E+4 /ml	Added to pasteurised whole fat milk	Added to pasteurised whole fat milk	-2.0 cfu per ml	ST67	77
		4h						-3.5 cfu per ml		
		8h						-2.5 cfu per ml		
Semi-skimmed raw milk	Cocktail (φA72 & φH5)	4h	37 °C	-	1E+5 /ml	Added to Semi-skimmed raw milk	Added to Semi-skimmed raw milk	-0.2 cfu per ml	ST67	77
		7h						-1.2 cfu per ml		
		11h						-1.7 cfu per ml		
Whole fat raw milk	Cocktail (φA72 & φH5)	4h	37 °C	-	1E+5 /ml	Added to whole fat raw milk	Added to whole fat raw milk	-1.2 cfu per ml	ST67	77
		7h						-2.2 cfu per ml		
		11h						-2.7 cfu per ml		

Table 16: Evidence table for the biocontrol of *Bacillus* in foods

Product	Phage name	Contact exposure conditions			Level of phage dose pfu per g	Method of food inoculation		Log reduction achieved per ml	Reference details	
		Time	Temperature	pH		Pathogen	Bacteriophage		ID	% Review score
Cheonggukjang (fermented soyabean paste)	JBP901	24 h	37 °C	-	1E+8 /g	Suspended in tryptose broth or peptone water prior to inoculation at a level of 10^3 cfu per ml	Suspended in SM buffer prior to inoculation	>5 log CFU /g (host suspended in tryptose broth) or <2 log CFU /g (host suspended in peptone water)	BA14	82
Cheonggukjang (fermented soyabean paste)	JBP901	12h	37 °C	-	1E+9 /g	Added to cheonggukjang at a level of 10^2 cfu per 7	Added to cheonggukjang	1.7-2.4 log CFU /g	BA37	75
		24h						1.7-2.4 log CFU /g		
		12h	37°C	-		Added to cheonggukjang at a level of 10^3 cfu per 7		1.8-2.4 log CFU /g		
		24h						1.5-2.2 log CFU /g		
Mashed potato	FWLBc1	24 h	25° C	-	1E+4 /g	Added to mashed potato	Added to mashed potato	<1 log CFU /g	BA35	82
Mashed potato	FWLBc2	24 h	25 °C	-	1E+4 /g	Added to mashed potato	Added to mashed potato	<1 log CFU /g	BA35	
Mashed potato	FWLBc1	24 h	25 °C	-	1E+8 /g	Added to mashed potato	Added to mashed potato	>6 log CFU /g	BA35	
Mashed potato	FWLBc2	24 h	25°C	-	1E+8 /g	Added to mashed potato	Added to mashed potato	>6 log CFU /g	BA35	
Mashed potato	FWLBc1	24 h	10° C	-	1E+4 /g	Added to mashed potato	Added to mashed potato	No effect	BA35	
Mashed potato	FWLBc2	24 h	10 °C	-	1E+4 /g	Added to mashed potato	Added to mashed potato	No effect	BA35	
Mashed potato	FWLBc1	24 h	4 °C	-	1E+4 /g	Added to mashed potato	Added to mashed potato	No effect	BA35	
Mashed potato	FWLBc2	24 h	4 °C	-	1E+4 /g	Added to mashed potato	Added to mashed potato	No effect	BA35	

Table 17: Evidence table for the biocontrol of Lactic Acid Bacteria in foods

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu/g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Spoilage Organism	Phage		ID	% Review score
Beer (4.3% ethanol v/v)	SA-C12	24, 48hr	30°C	4.4	10^8 pfu/ml	20-ml aliquots of filter sterilised beer inoculated with 10^2, 10^3 or 10^4 cfu/ml,	20-ml aliquots of inoculated beer treated with phage	Removal to below LOD: by 24hr for 10^2 (L.brevis) by 48 hr for 10^3 & 10^4	LA45	65

Table 18: Evidence table for the biocontrol of Enteric bacteria in foods

Food product	Phage name	Contact exposure conditions			Level of phage dose pfu per g	Method of food inoculation		Log reduction achieved per unit	Reference details	
		Time	Temperature	pH		Spoilage organism	Phage		ID	Review score
UHT milk	Cocktail 3 phage – ATCC25922	3h	25°C	n/a	1.0 x 10^9 per ml	1.0 x 10^5 per ml	n/a	>4.0	ES83	73
		9h						>4.0		
		24h						>4.0		
	Cocktail 2 phage – *E. coli* O5H-	3h	25°C					>4.0		
		9h						>4.0		
		24h						>4.0		
	Cocktail 3 phage – ATCC25922	24h	5-9°C	n/a	1.0 x 10^9 per ml	1.0 x 10^5 per ml	n/a	>4.0		
		96h						>4.0		
		168h						>4.0		
	Cocktail 2 phage – *E. coli* O5H-	24h	5-9°C					>4.0		
		96h						>4.0		
		168h						>4.0		
Raw milk	Cocktail 3 phage – ATCC25922	3h	25°C	n/a	1.0 x 10^9 per ml	1.0 x 10^5 per ml	n/a	>4.0		
		9h						>4.0		
		24h						>4.0		
	Cocktail 2 phage – *E. coli* O5H-	3h	25°C					>4.0		
		9h						>4.0		
		24h						0.0		
	Cocktail 3 phage – ATCC25922	24h	5-9°C	n/a	1.0 x 10^9 per ml	1.0 x 10^5 per ml	n/a	>4.0		
		96h						>4.0		
		168h						>4.0		
	Cocktail 2 phage – E. coli O5H-	24h	5-9°					0.6		
		96h						>4		
		168h						1.8		

4 DATA ANALYSIS

An in depth review of data was carried out for each of the individual organisms that were entered into the evidence tables, to determine:

i) Efficacy of bacteriophage treatments in foods; and
ii) Pin point any safety issues on the use of bacteriophage in foods quoted in the literature.

Efficacy of bacteriophage treatments

The potential of bacteriophage as effective biocontrol agents in foods can be assessed by a range of criteria including:

- Food products that the bacteriophage treatments are compatible with;
- Specificity of bacteriophage;
- Limitations on the use of bacteriophage on foods;
- Possible issues arising from the use of bacteriophage e.g. the emergence of bacteriophage resistant organisms;
- The ability of bacteriophage to protect against product recontamination.

The data collated in the final evidence tables (listed in the report and appendix 3), was evaluated using the above listed criteria to establish the suitability of bacteriophage treatments to control microbial contamination on foods.

Foods compatible with bacteriophage treatments

Due to the significantly higher volume of information gathered for *L. monocytogenes*, *Salmonella* spp., and STEC, it was decided to have dedicated sections for each of these organisms. The remaining pathogens and spoilage organisms are then covered in subsequent sections.

Listeria monocytogenes

The analysis for *L. monocytogenes* involved 27 individual food types reported in 15 separate studies. Six food groups were involved in the evaluation of bacteriophage performance (meats, fish and seafood, dairy, fresh produce, liquids and composite meals). Of the 6 food groups used with *L. monocytogenes*, the predominant one was meat with 8 different products listed. A summary of the data obtained from the bacteriophage treatment of foods is shown in table 19. The data takes into account food categories and level of bacteriophage treatment (given as 'multiplicity of infection' (MOI), with minimum and maximum log reductions).

Table 19: Summary of information from the evidence tables for *L. monocytogenes*

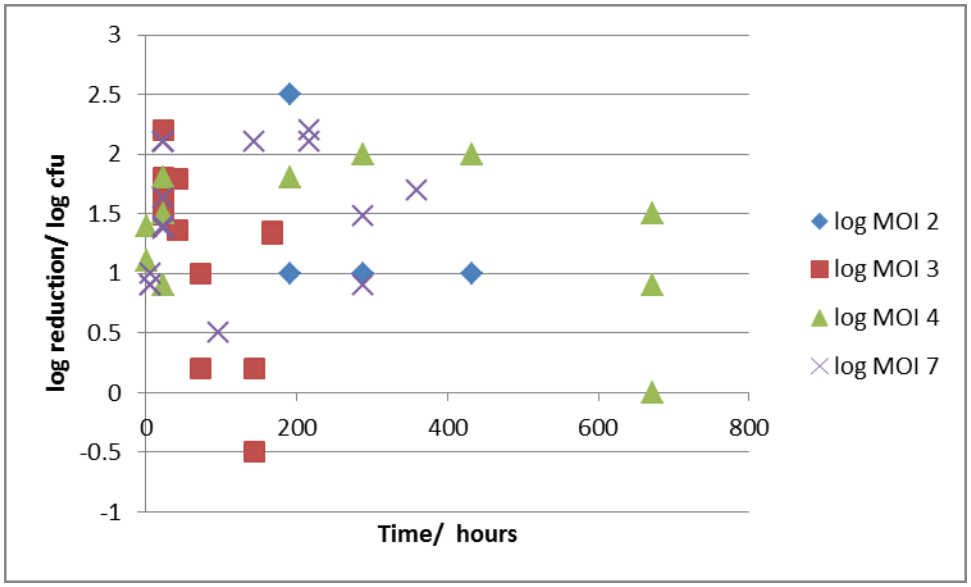
category	subcategory	log MOI	Min log reduction (cfu)	Max log reduction (cfu)	Time points (hours)	No of papers
Meat	Cooked	2	1	2.5	192, 288, 432	1
		3	-0.5	2.2	24, 42, 72, 168, 144, 168	2
		4	0.9	2.0	0.5, 24, 192, 288, 432	2
		7	0.5	2.2	24, 96, 144, 216, 288, 360	1
	Processed	3	1.18	2.16	0.33, 24, 42, 72, 144, 168, 240	3
		4	-3.5	0.5	72, 168, 336, 672	1
Produce	Fruit	1	-3.8	2	24, 48, 72, 120, 192	2
	Salad	3	0.5	2.8	0.083, 24, 48, 72	2
	Vegetables	3	1.0	3.0	24, 48, 72	1
Dairy	Soft cheese	2	0.8	2.3	0.5, 198	1
		3	1.2	5.6	1, 4, 24	1
	Hard pasteurised cheese	2	0.5	0.7	0.083,24, 48	1
	Cheese brine	3	3.0	3.0	24, 72, 144, 312	1
liquids	Fruit juice	3	-0.1	4.7	48, 120,192	1
	Flavoured milk	3	2.2	3	24, 72, 144, 312	1
	ESL milk	1	4.0	4.0	192	1
	ESL milk	2	6.0	6.0	192	1
Fish and shellfish	Smoked fish (salmon)	0	0.0	0.4	24	1
		1	1.0	1.0	24	1
		3	0.0	2.5	24,72,144	1
		4	2.5	3.0	24	1
	Seafood	3	0	3	24, 72, 144	1
	Raw	3	0.2	2.1	0.5, 96, 240	1
		4	2.7	3.5	0.5,2	1
		5	2.4	3	0.5,2	1
		6	1.6	1.8	0.5,2	1
Other	Composite meals	3	0.62	3.0	24,42,168	2

Further analysis was carried out for each food group listed in Table 19, to highlight trends in the data, to determine the factors influencing bacteriophage performance, and to draw out any limitations on the use of bacteriophage to control *L. monocytogenes* in foods.

Cooked Meat

The log reduction in *L. monocytogenes* achieved by the bacteriophage treatments ranged from -0.5 to 2.5 in cooked meat and represents data reported for cooked turkey, beef and chicken. A summary of the data collated is shown in Figure 2 which shows the log reduction against time for each of the log MOI values. One of the key observations in these studies has been that high concentration of bacteriophage is needed to deliver a significant reduction in host cell numbers. The results from (Bigot et al. 2011) showed that with MOI of log 2 the reduction in *L. monocytogenes* was around 1 log cfu per cm^2, whereas at log MOI log 4, the log reduction was approximately 2.5 log cfu per cm^2.

Figure 2: Data from review for reduction of *L. monocytogenes* on cooked meat products with various levels of bacteriophage treatment.



Studies by Guenther et al. 2009 acknowledged the impact of food matrix on the efficacy of bacteriophage treatment and stated that the contact time should allow passive diffusion of the bacteriophage through food taking into account spatial limitations. One of the main barriers to movement of bacteriophage in a solid matrix such as meat is the large surface area and the ability of the food to absorb liquids. In addition, the target organism may be embedded in the food structures which would prevent contact with the bacteriophage. The efficiency of bacteriophage treatments can be improved to overcome the issues associated with solid foods by using larger numbers of bacteriophage, using bigger volume of bacteriophage or by application of bacteriophage prior to contamination.

The same authors recommended that bacteriophage should be applied at a concentration of 10^8 pfu per g or per cm^2 for optimum efficiency and that application should be specifically optimised for the food system.

The mode of bacteriophage application was also investigated by Anany et al. (2011). They reported that immobilisation of the bacteriophage onto a modified cellulose membrane allowed the positively charged bacteriophage tail to better attract negatively charged bacteria. One of the key benefits of immobilisation is that it reduces the potential for non-specific binding that can reduce the efficacy of the bacteriophage.

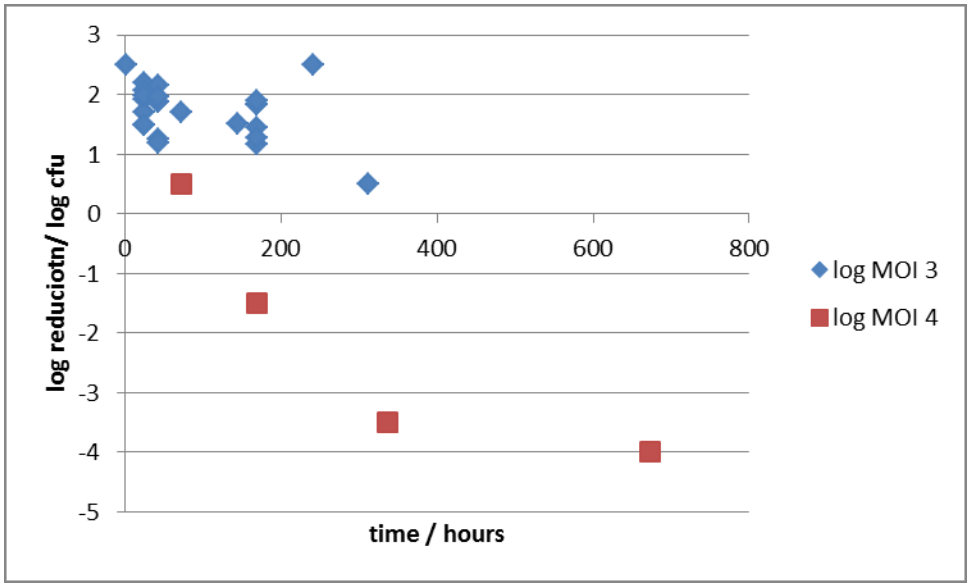
Other interesting points of note were that the packaging conditions had an impact on the level of log reduction of *L. monocytogenes* obtained over the life of RTE turkey. The immobilised cocktail of phage resulted in a greater reduction in *L. monocytogenes* in samples packed under modified atmosphere packaging (MAP) or vacuum packed compared to aerobically stored samples (Anany et al. 2011).

Key finding 13: Bacteriophage are capable of delivering up to a 2.5 log reduction in *L. monocytogenes* on cooked meat. The activity of the bacteriophage has been shown to be enhanced when meat is packed using MAP or vacuum packing.

Processed meat

Studies involving processed meats involved a selection of products including sausages, ham and fermented meats. Analysis of the results reported for processed meat revealed that bacteriophage delivered between -3.5 and a 2.16 log reduction in *L. monocytogenes*. The results for processed meat are summarised in Figure 3. Data indicates that initial reduction in *L. monocytogenes* remains over a 200h period at a MOI of log 3, however after that it is possible that some re-growth may occur. This was highlighted in a study by Holck and Berg (2009), where although the level of *L. monocytogenes* in the bacteriophage treated samples was lower than the untreated control, growth still occurred during long term storage of ham.

Figure 3: Data from review for reduction of *L. monocytogenes* on processed meat products with various levels of bacteriophage treatment.



As with the cooked meat, there are physical characteristics of the food matrix (processed meat) that reduce the level of passive diffusion that occurs. There is also the potential for the target organism to

be located inside food structures, hence limiting contact with the bacteriophage. As with the cooked meat, adjustments can be made to overcome some of these issues. Work by Anany et al. (2011) recommended that bacteriophage should be applied at a concentration of 10^8 pfu per g or per cm^2 for optimum efficiency. Optimisation of application for bacteriophage should be done for each food to ensure its best performance.

Key finding 14: The application of bacteriophage can deliver a 2 log reduction in *L. monocytogenes* on processed meat.

Produce

There were 3 papers selected during the review which explored the decontamination of *L. monocytogenes* on fresh produce covering fruit, salad vegetable and vegetables using bacteriophage. Data collected during the review indicated that bacteriophage has limited success in controlling *L. monocytogenes* on fresh fruit, with all 3 fruit types showing growth over storage (Oliveira et al. 2014). The authors from this study indicated that the performance of bacteriophage was influenced by the pH and the physical form of the food matrix. For salad vegetables, studies reported up to a 2.2 log reduction with the use of a commercially produced cocktail of 6 bacteriophages which was applied at a MOI of log 3 compared to the fruit where an MOI of log 1 was used.

Key finding 15: Bacteriophage did not effectively control the level of *L. monocytogenes* on some fruit; however they were successful in lowering the numbers of *L. monocytogenes* on salad vegetables and vegetables by 1-2 log.

Dairy

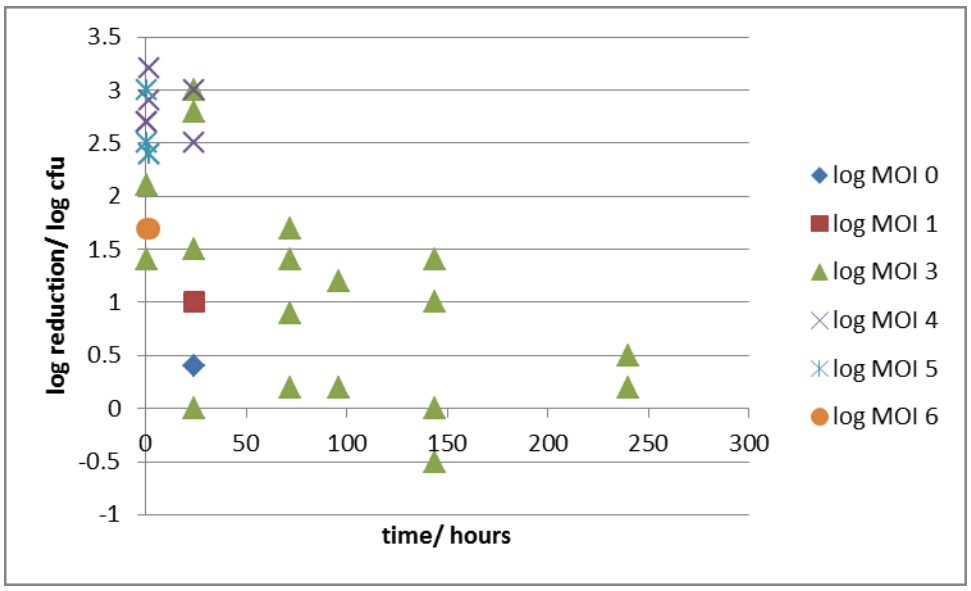
Analysis of the data for cheese indicated that greater log reductions in *L. monocytogenes* were obtained with soft cheese compared to hard cheese at a MOI of log 2. Studies by Silva et al. (2014), report an initial log reduction of 2.0 which decreases over storage suggesting that re-growth has occurred. Other observations noted in the same study were that the performance of the bacteriophage is dependent upon the level of *L. monocytogenes*, and that a high level of bacteriophage is needed to ensure sustained inactivation of pathogen over storage. At an MOI of log 3 the log reductions in *L. monocytogenes* were greater in soft cheese than at a MOI of log 2.

Key finding 16: Data suggests that bacteriophage are more effective at reducing *L. monocytogenes* in soft cheese compared to hard cheese, although high levels of bacteriophage are required to control numbers over long term storage.

Fish and seafood

The results for the fish and seafood category showed that a 0 to 3.5 log reduction was achieved in *L. monocytogenes* following the application of bacteriophage onto the samples. Details of the bacteriophage performance at different log MOI values are shown in Figure 4.

Figure 4: Data from review for reduction of *L. monocytogenes* on fish and seafood products with various levels of bacteriophage treatment



There were 3 papers focussing on the use of bacteriophage at varying MOI log values in smoked salmon. Analysis of the data revealed that the higher the log MOI used on smoked salmon, the greater the log reduction was achieved. This was also true for the raw fish with an optimum MOI of log 4 for the greatest log reductions obtained in research to date. Other limitations reported in the literature were that bacteriophage activity was temperature dependant although this was refuted in other published work.

Research has explored the use of combination treatments of bacteriophage combined with the GRAS approved antimicrobials nisin and lauric arginate (Kamlesh et al. 2014). The results obtained showed co-application of nisin and bacteriophage was able to reduce *L. monocytogenes* to below level of detection. Despite this promising result, the authors felt that nisin and bacteriophage could not be used practically, as nisin targets all Gram positives and the bacteriophage would be host specific. Further issues raised were that most of the studies carried out used laboratory isolates rather than those commonly found in the processing environment, therefore their true efficacy in the food industry is yet to be elucidated (Soni and Ramakrishna et al. 2010).

Key finding 17: Bacteriophage at a level of MOI log 4 were able to lower the level of spiked *L. monocytogenes* by up to 3 logs in fish and seafood.

Liquids

Limited data has been published with only 3 papers in this product type covering fruit juice and milk based products. Unlike their effects on fruit, the bacteriophage was reported to deliver up to a 4 log reduction in *L. monocytogenes* in juice. One of the explanations for this would be the greater mobility of the bacteriophage in the liquid compared to solid matrices. Initial data from the milk study revealed that bacteriophage performance appeared to differ between strains and that each one needs to be optimised to ensure best performance (Rodriguez-Rubio et al. 2015).

Key finding 18: Bacteriophage have been shown to effectively reduce the level of *L. monocytogenes* in fruit juice by up to 4 log and in milk by up to 6 log.

***Salmonella* spp.**

In the papers reviewed, a total of 26 different individual food products were analysed from 18 separate reports. Four food groups were involved in the assessment of *Salmonella* bacteriophage performance (meats, fresh produce, liquids and dry foods). Of the 4 food groups examined for *Salmonella* spp, the predominant one was meat with 24 different products listed. The papers reviewing bacteriophage treatment of foods against *Salmonella* are summarised in Table 20. Data is described in terms of food categories, and level of phage treatment given as multiplicity of infection (MOI), with the minimum and maximum log reductions given.

Table 20: Summary of the data from the evidence tables for *Salmonella* spp

Category	Subcategory	Log MOI	Min log reducrion (cfu)	Max log reduction (cfru)	Time points (hours)	No of papers
Meat	Raw meat	3	0.7	2.2	24, 48, 120, 168	1
		4	1.75	1.75	24	1
		7	1.4	2	24, 48	1
	Meat skin	<1	-0.2	0.4	1, 48, 96	1
		1	0	3.5	1, 48, 96	1
		2	0.5	3.5	1, 48, 96	1
		3	1	3	0.5, 1, 24, 48, 96, 168	3
		4	0.4	5.2	1, 24, 48, 96	3
	Cooked meat	4	2	2	24	1
		5	3	3	48	1
		7	1.73	1.83	24, 48	1
	Mixed seafood	8	1.9	1.9	48	1
Fresh produce	Vegetables	4	1.9	3.9	0.5, 1, 48	2
		8	2.79	2.79	24	1
	Sprouted seeds	4	2	6.8	24	2
		6	3.4	3.4	96	1
	Fruit	8	0.49	1.78	24	1
Liquids	Eggs	3	0.9	0.9	2	1
		4	1.2	2	24, 48, 72	1
		8	0.8	2	24, 48	2
	Milk	1	0.5	6	3, 6, 9, 24	1
		2	6	6	3	1
		8	1.7	4.5	24, 48	2
	Drinks	8	2.1	2.5	24, 48	1
Dry food	Pet food	5	0.75	0.75	1	1
		6	1.37	1.37	1	1

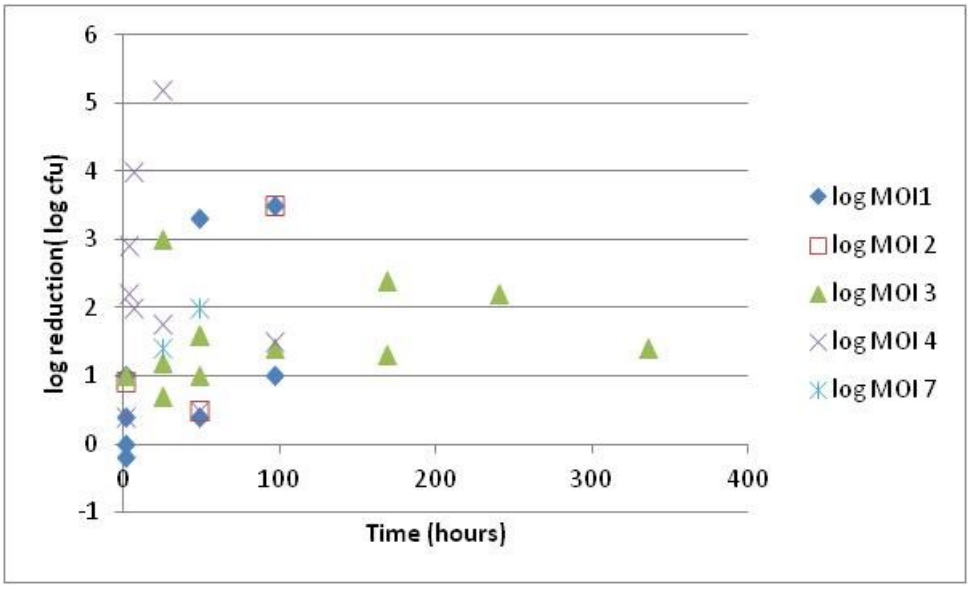
Category	Subcategory	Log MOI	Min log reducrion (cfu)	Max log reduction (cfru)	Time points (hours)	No of papers
		7	2.05	2.05	1	1

More detailed analysis was carried out for each food group listed in Table 20 to highlight trends in the data, to determine the factors influencing bacteriophage performance and to draw out any limitations on the use of bacteriophage to control *Salmonella* spp in foods.

Meat products

The minimum and maximum log reduction achieved at different MOI for various treatment times are shown for the raw meat products in Figure 5. Data revealed that the reductions in *Salmonella* observed, ranged from 0 to 5.2 logs. The data suggests that MOI of equal to or greater than log 3 are required to show reductions greater than 1 log in levels of *Salmonella*. Evidence showed that bacteriophage treatments with MOI of equal to or lower than log 2 on pig skin gave greater than 1 log reduction in *Salmonella* when the treatment time was at least 48 hours (Hooton et al. 2011). It was postulated that the low log reductions given at the low MOI show that the therapeutic effect of the bacteriophage is passive. Passive activity requires a higher dose of bacteriophage to enable a reduction in microbial levels in the absence of bacteriophage replication. The advantage of the passive effect is that it is less likely to result in the development of bacteriophage resistant bacteria.

Figure 5: Data from review for reduction of *Salmonella* on raw meat products with various levels of phage treatment.



Reductions of 1.4 to 2 logs were shown in raw chicken mince treated with bacteriophage at a MOI of log 7 over 24 and 48 hours. For the cooked meat products the levels of bacteriophage treatment reported were between MOI log 4 and 7, over 24 hours. Over 48 hours, the log reductions were between 1.73 and 3 (Zinno et al. 2014, Guenther et al. 2012, Turner 2013).

Hungaro et al. (2013) compared a bacteriophage treatment using a cocktail of 5 bacteriophages on chicken skins (MOI 4 log for 30 mins) with treatments of: lactic acid (2% for 90 seconds); peroxyacetic acid (100ppm/10min); and dichloroisocyanurate (200ppm/10min). None of the treatments were found

to be statistically different, suggesting that the bacteriophage treatment is equivalent to those treatments.

It has been suggested that higher doses of bacteriophage are required for treatments at lower temperatures, due to the slower rate of *Salmonella* growth at chill temperatures and therefore the ability of the bacteriophage to replicate (Spricigo et al. 2013 and Hooton et al. 2011). This work was carried out with meats and so most of the meat trials were carried out at chill temperatures due to the nature of the products.

Key finding 19 : Bacteriophage are capable of reducing *Salmonella* contamination on raw meat by up to 3 logs and were shown to be equivalent to alternative treatments including lactic and peroxyacetic acid.

Fresh produce

Data for the fresh produce studies experiments with salad leaf products, sprouted seeds, broccoli and fruits such as strawberries, cantaloupe melon and tomatoes. The salad leaf products were all tested with cocktails of 3 bacteriophage strains at levels of MOI at log 4, over treatment periods of 0.5 to 24 hours. The reductions observed were between 1.9 and 3.9 logs (Spricigo et al. 2013 and Turner 2013).

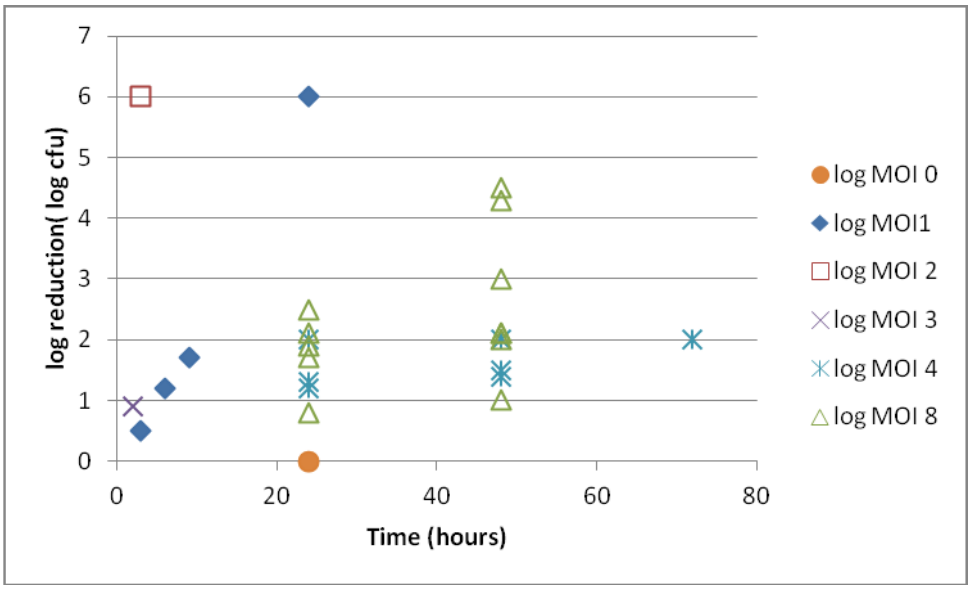
Sprouted seeds were treated with cocktails of 3 bacteriophage strains. A trial on beansprouts with an MOI dose of log 4 over 24 hours produced a 2 log reduction (Turner 2013), whereas a trial on mung beans with an MOI of log 6 over 96 hours produced a 3.4 log reduction (Jianxiong et al. 2010). Trials on fresh produce with MOI of less than log 4 gave no reduction in the number of *Salmonella* present (Jianxiong et al. 2009). The lack of 'kill' of *Salmonella* was thought to be due to the relatively low MOI in combination with the storage temperature of 15°C. It was suggested that a higher MOI and an increase in storage temperature from 15°C to 30°C would have resulted in a greater log reduction of *Salmonella*. High MOI doses of log 8 over 24 hours produced reductions of 2.79, and 3.69 in broccoli and cantaloupe respectively, but only of 0.49 in strawberries over 24 hours (Magonne et al. 2013).

Key finding 20: Bacteriophage treatments were able to effectively reduce *Salmonella* contamination on a selection of fruit and vegetables by up to 6.8 logs with the exception of strawberries.

Liquid products

The data for the liquid products included trials carried out on eggs, milk, and soft drinks including apple juice and energy drinks. The minimum and maximum log reduction achieved at different MOI for various treatment times are shown for all the liquid products in Figure 6. The range of the reductions in *Salmonella* observed was 0 to 4.5 logs. The data suggests that lower MOI doses are more successful for liquid products than for solids, however the MOI of equal to or greater than log 1 were required to achieve reductions of 1 log in levels of *Salmonella*. For the liquid egg products MOI equal to or greater than 4 being required to achieve the same reduction in *Salmonella*. It was thought that this was possibly due to the more viscous nature of the product. Treatments of milk and soft drink products with MOI of log 8 for 24-48 hours resulted in count reductions of 1.7 – 4.5 logs.

Figure 6: Data from review for reduction of *Salmonella* in liquid products with various levels of phage treatment.



Some of the studies suggested that in solid food samples the virus particles become immobilised on the food surface after 24h, which restricts their ability to come into contact with non-infected *Salmonella.* The lack of contact of the bacteriophage with the remaining *Salmonella* could allow the cells to grow over further storage (Geunther et al. 2012, Spricigo et al. 2013, and Tranuk et al. 2014). This could also explain the greater reductions observed with lower MOI in liquid products, where the bacteriophage can diffuse freely, and the requirement of higher MOI with the more viscous liquid egg products.

Key finding 21: Bacteriophage treatment of milk and soft drink products with an MOI of log 8 resulted in a reduction in *Salmonella* of up to 4.5 log.

STEC

In the papers reviewed for STEC, a total of 23 different food products were analysed from 14 separate reports. The 23 food products were grouped into 3 types: meat; fresh produce; and liquids. The papers reviewing bacteriophage treatment of foods against STEC are summarised in Table 21.

Table 21: Summary of information from the evidence tables for STEC

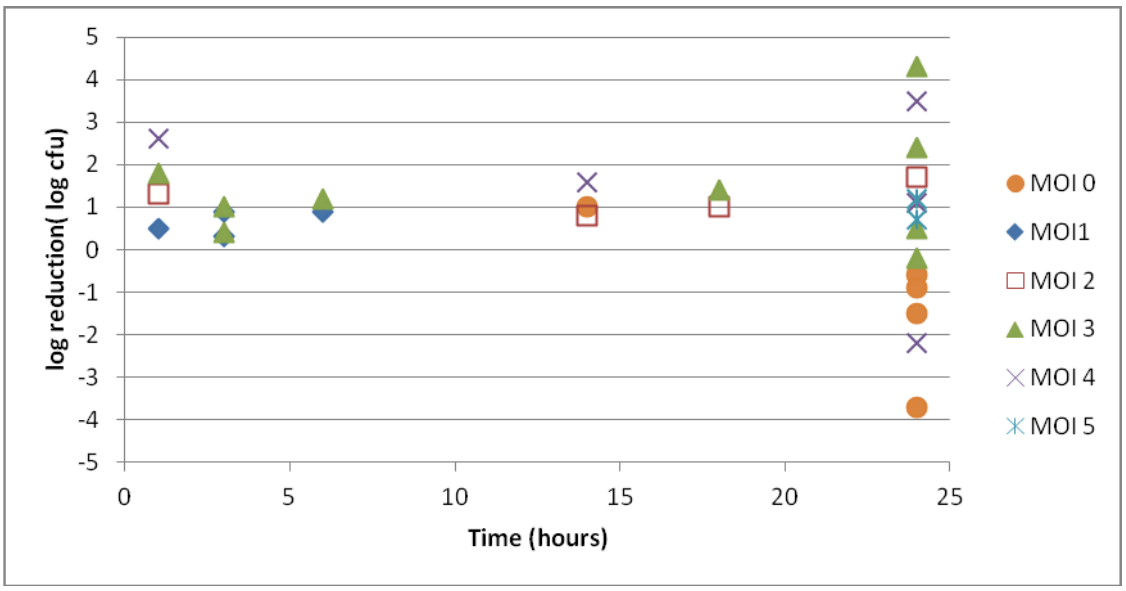
Category	Sub category	Log MOI	Min log reduction (cfu)	Max log reduction (cfu)	Time points (hours)	No of papers
Meat	Raw meat	0	-3.7	1	14, 24	2
		1	0.3	0.9	1, 3, 6, 144	2
		2	0.8	1.3	1, 14, 18	1
		3	-0.2	2.4	1, 3, 6, 18, 24, 144	3
		4	-2.2	2.6	1, 14, 24	2
		5	1.2	1.2	24	1
	Meat skin	4	3.5	4.4	24, 120	1
	Cooked meat	0	-1.5	-0.9	24	1
		2	1.7	1.7	24	1
		3	4.3	4.3	24	1
		4	1.1	1.1	24	1
		5	0.7	0.7	24	1
Fresh produce	Vegetables	0	0.16	3.68	0.17, 1 , 24	1
		1	0.56	3.21	0.17, 1 , 24	1
		2	0.41	3.78	0.17, 1 , 24	2
		3	1.92	4	0, 2, 24, 48, 360	3
		4	4	4	3	1
	Fruit	2	0.53	0.53	3	1
		3	0.74	0.74	3	1
		4	0.21	2.97	0, 48, 120, 168	1
Liquids	Milk	3	0.85	0.85	24	1
		4	2	3	1	2
		5	3	3	1	1
	Juice	4	1	1	3	1

Further analysis was carried out for each food group listed in Table 21 to highlight trends in the data and to determine the factors influencing bacteriophage performance. The analysis was also used to draw out any limitations on the use of bacteriophage to control STEC in foods.

Meat products

Figure 7 shows the minimum and maximum log reductions achieved at different MOI for various treatment times up to 24 hours in meat products. The majority of trials were carried out on raw beef; however some trials used cooked beef and one trial on chicken skin. Some of the trials gave a negative log reduction meaning that the levels of STEC increased. Where an actual reduction was recorded these ranged from 0.3 to 4.4 logs. The data suggested that an MOI of greater than log 2 is required to produce a reduction of greater than 1 log, and an MOI of greater than log 3 is needed to achieve a reduction of greater than 2 log.

Figure 7: Data from review for reduction of STEC from on meat products with various levels of phage treatment up to 24 hours



Where an increase in STEC levels was observed, this was always in trials that had been carried out at a storage temperature of 24°C over 24 hours. Storage at 24°C allowed STEC to grow post-treatment with bacteriophage. However the growth was slower than that in non-treated controls (Hudson et al. 2015). Most of these treatments were also with a low MOI of log 0 meaning that the level of bacteriophage was the same as the level of target organism. These trials also reported that the higher MOI-treated samples stored at 24°C for 24 hours displayed grow back of STEC, however this was at a slower rate. Trials in the same study conducted at 5°C showed that the reduction was proportional to the concentration of phage added. One study suggested that the lytic cycle of the bacteriophage could be initiated when the food (in this case chicken skin), was still at room temperature prior to chill storage (Lopez-Cuevas et al. 2012). The authors recommended that more effective reduction would be obtained if the bacteriophage preparation could be added directly post slaughter to allow for immediate bacterial lysis to occur. Work by (Liu et al. 2015) found that overall inactivation increased with incubation temperature, MOI and duration of treatment. They reported that chill storage did not prevent the bacteriophage infecting the target cells but may have delayed the bacterial lysis. This was corroborated by findings in Hudson et al. 2013.

Hudson et al. 2015 suggest that there is a minimum concentration of bacteriophages required for any effect to be observed of log 4 to 5 per cm^2 regardless of MOI, and that MOI is not relevant to solid foods. However if an MOI of 4 or 5 is required to target foods containing 1 to 2 log of STEC, then the minimum bacteriophage concentration would be adhered to. Other papers suggested that higher MOI doses increased the likelihood of individual bacteria contacting at least one bacteriophage. (Liu et al. 2015, Anany et al. 2010, Tomat et al. 2013 and Viazis et al. 2011).

Anany et al. 2011 reported a trial in which they used bacteriophage immobilised on a membrane and then used as packaging for raw beef products as a treatment. The authors suggest that being immobilised on a membrane would enhance the capture efficiency by reducing the amount of nonspecific binding to the food matrix. The charge difference between bacteriophage ‘heads’ and ‘tail’ fibers was used to specifically immobilise a cocktail of bacteriophages through their head, leaving the

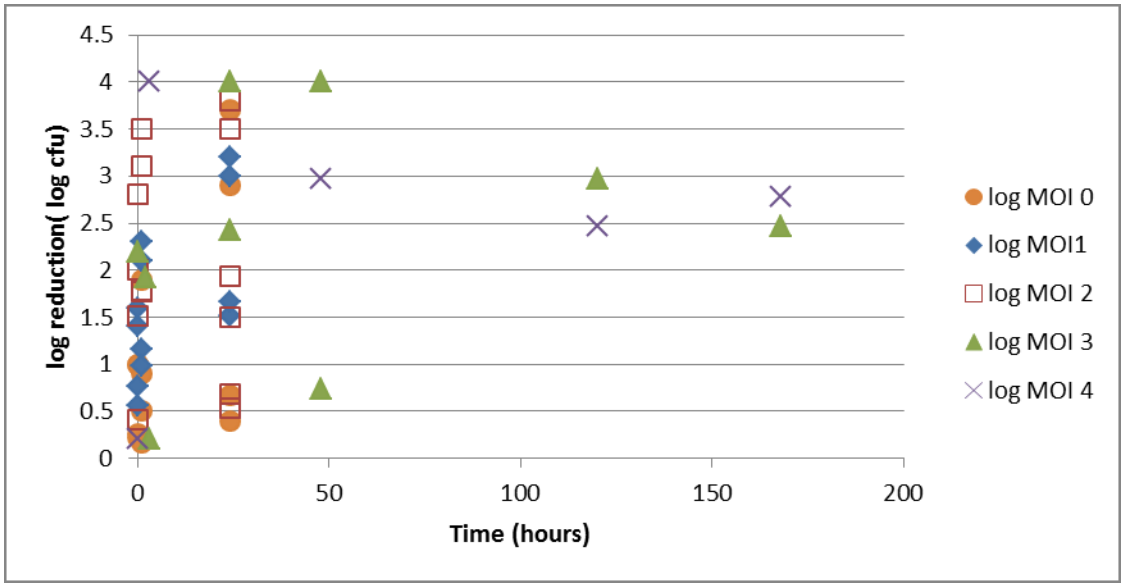
tail fibres free to capture and infect the target bacteria. A reduction of 1.2 log of a 2.2 log load was achieved after 1 day at chill temperatures.

Key finding 22: Bacteriophage treatments are able to reduce the level of STEC on meat by up to 4 log. Evidence suggests that intervention directly post slaughter may provide the greatest efficacy of bacteriophage inactivation of STEC.

Fresh produce

The data for the fresh produce studies included experiments with vegetables (such as lettuce, spinach, cabbage, and broccoli) and fruits (such as strawberries and cantaloupe). The minimum and maximum log reductions achieved at different MOI for various treatment times are shown in Figure 8.

Figure 8: Data from review for reduction of STEC on fresh produce products with various levels of phage treatment.



The vegetable products were mainly treated with cocktails of 3-8 bacteriophage strains, with only 1 using a single bacteriophage treatment. The levels of MOI ranged from log 0 to 4 and were used over treatment periods of 0.5 to 48 hours with the exception of 1 trial which tested after 15 days. The reductions observed were between 0.16 and 4.0 logs (Giddings 2012, Viazis et al. 2011, Sharma et al. 2009, Jitendra et al. 2011, Young Duck et al. 2013, and Magnone et al. 2013).

Treatments on fruit were described in two trials. One study evaluated a cocktail of 3 bacteriophages used to treat strawberries and cantaloupe (inoculated with 5 STEC strains) at MOI of log 2 and 3 respectively. After 24 hours the reductions observed were 0.53 – 0.74 log cfu (STE94). The second study described the decontamination of cantaloupe inoculated with a single STEC isolate treated with a cocktail of bacteriophage (at log MOI ranging from 2 – 4), over time periods of 0 to 7 days at chill temperatures. Analysis of the data revealed that log reductions up to 2.97 log cfu were achieved (Sharma et al. 2009). The same trials were also carried out at 20°C. An increase in the levels of STEC occurred on both bacteriophage treated samples and controls stored at 20°C, although the levels of STEC were more than 1 log lower on the treated samples compared to the controls, showing that the treatment had an effect. In a trial where ranges of MOI, temperature and exposure time of the

treatment were investigated, it was found that rates of STEC reduction generally increased with increasing MOI, temperature and exposure time. (Viazis et al. 2011)

The method for bacteriophage application for each trial is given in the evidence tables in the Appendix 2. Sharma et al. 2009 suggested that method of applying the treatment is crucial to success, with spray treatment more effective than immersion. They reported that the spray method improved contact between the bacteriophage and the target organism.

A study carried out on baby spinach and lettuce, used bacteriophage treatment alone and in combination with the essential oil extracts (0.5% trans-cinnamaldehyde). The single treatments gave a reduction of 3 and 1 log cfu/leaf at temperatures below 8°C after 24hours, whereas the combination treatments produced a 5 log reduction to below the limit of detection after 10 minutes and 1 hour at temperatures equal to and greater than 4°C for spinach and lettuce respectively. The authors suggested that the action of the essential oil on the membrane fatty acid of the STEC, could allow the bacteriophage to attach to the membrane more readily allowing for faster infection. (Viazis et al. 2011).

Another combination treatment using bacteriophage followed by a produce wash was trialled on broccoli, cantaloupe and strawberries. The products were treated with the bacteriophage for 24 hours and then subjected to a levulinic acid produce wash for 1 or 5 minutes. The results with co-treatment of bacteriophage treatment and produce wash were the same as the produce wash alone, which was a reduction of 4.1 to 4.6 logs to below the limit of detection. It is therefore not possible to see if the bacteriophage treatment had any additional effect. The trials showed a possibility of using a combined treatment of produce wash with bacteriophage treatment to mitigate the risk of potential pathogen growth post treatment (Magnone et al. 2013).

Key finding 23: Studies indicate that bacteriophages are capable of reducing the level of STEC on fresh produce by up to 4 logs on vegetables, 2.97 logs on fruit and 3 logs on salad leaves. The mode of application of bacteriophage treatment may also be important, with spray treatments reported to be more effective than immersion treatments.

Liquids

The data for the liquid products mainly included two trials carried out on milk (sterilised and fermented) and a trial carried out on vegetable juice. The range of the reductions observed was 0.85 to 3 logs. The MOI doses used ranged from log 3 to 5 and suggested that a MOI of 4 or greater produced log reduction of 1 or more over treatment times of 1 to 3 hours. A lower reduction was observed with a MOI of 3 over 24 hours.

It is important to remember that results will vary with the product type being treated. Factors such as food type, nutrient component and surface type could have an effect. In order to have an effect on the bacteria the bacteriophage needs to bind to receptors on the target bacteria. Bacteriophage adsorption could be inhibited by various conditions such as pH. The low pH of some products could prevent attachment of bacteriophage to host cells preventing infection (Young Duck et al. 2015 and Lopez-Cuevas et al. 2012).

Key finding 24: Limited data is available on bacteriophage treatments of STEC in liquids. However, available data suggest that MOI of log 4 can deliver at least a 1 log reduction in STEC.

Staphylococcus aureus

Data analysis for *S. aureus* involved 5 separate studies covering the dairy products cheese and milk. A summary of the data obtained from the bacteriophage treatment of foods with regards to food categories and level of bacteriophage treatment (given as multiplicity of infection (MOI)), with the minimum and maximum log reductions is shown in Table 22.

Table 22: Summary of information from the evidence tables for *S. aureus*

Category	Subcategory	log MOI	min log reduction (cfu)	max log reduction (cfu)	Time points (hours)	No of papers
Dairy	Hard cheese	0	>4.0	>4.0	6, 336	1
		1	>4.0	>4.0	24, 720	1
		2	>4.0	>4.0	336	1
		3	>4.0	>4.0	336	1
	Pasteurised full fat milk	-3.5	-1.5	6.0	2, 4, 8	1
		-1.5	-3.0	0.0	2, 4, 8	1
		1	6.0	6.0	2, 4, 8	1
		2	-0.5	3.0	4, 24, 48	1
		2.5	-3.5	-2.0	2, 6, 8	1
		3	-1.0	4.0	2, 4, 8	1
		4	0.2	4.0	4, 24, 48	1
	UHT full fat	2.5	-1.7	0.0	2, 6, 8	1
	raw semi skimmed	2.5	-1.7	-0.2	4,7, 12	1
	raw full fat	2.5	-2.7	-1.2	4, 7, 12	1

Additional analysis was carried out for each food group listed in Table 22 to highlight trends in the data, to determine the factors influencing bacteriophage performance, and to draw out any limitations on the use of bacteriophage to control *S. aureus* in foods.

Data gathered for *S. aureus* revealed that bacteriophage added to milk prior to cheese production were capable of achieving at least a 2 log reduction in hard cheese after 24h at an MOI of log 1 and greater than 5 log reduction after 720h storage at 11°C (Bueno et al. 2012). Other studies also showed that *S. aureus* could be controlled with 2 different cocktails of bacteriophage introduced into the milk supplemented with calcium chloride, where numbers of the organism were lowered by over 4 logs after incubation for 336h (El Haddad 2014). Results from El Haddad (2014) indicated that MOI of logs 2 and 3 could deliver the same reduction in *S. aureus*.

The data obtained on the control of *S. aureus* on milk revealed a great variation in bacteriophage performance. One of the reasons for this was the use of 2 different incubation temperatures in one of the studies included in the review (Obeso et al. 2010). When the bacteriophage was added at MOI of 1, a log reduction was achieved at both 37°C and 15°C, whereas at higher MOI values, the bacteriophage required a longer contact time of 8h to achieve a 4 log reduction. Evidence also indicated that bacteriophage were not capable of delivering a reduction in the numbers of *S. aureus* in UHT full fat, raw full fat and raw semi skimmed milk.

Key finding 25: Bacteriophage are capable of reducing the level of *S. aureus* in cheese by up to 5 logs during storage for 24-336h.

Key finding 26: The ability of bacteriophage to reduce *S. aureus* in milk is dependent on the type and is influenced by storage temperature and MOI.

Spoilage organisms

Of the 9 representative spoilage organisms included in the review only 3 (*Bacillus* spp., lactic acid bacteria and the Enterics) were taken through to the final evidence tables. Most of the papers collated in this work failed to meet the criteria of the critical review process. The main reason for this was that the scores given in the critical review were below the required threshold of 40%. This suggests that insufficient details were given in the papers for entry into the evidence tables, and to undergo the systematic review process.

There were 3 *Bacillus* papers taken forward to the final evidence tables which covered 2 food groups: fermented foods; and composite foods. Evidence revealed that bacteriophage added to the Korean fermented bean paste 'Cheonggukjang' were capable of limiting the growth of *B. cereus* during fermentation (compared to the controls) by between 2.2 and at least 5 logs over a 24h period at MOI of log 5 to 7 (Nadeeka et al. 2014 and Haldong et al. 2011).

The majority of the research on *Bacillus* has been carried out in the composite food – mashed potato. Data generated for the impact of bacteriophage on *B. cereus* suggested that MOI of log 2 was successful in lowering the initial level of the spiked organism by more than 6 logs. When a lower MOI of log -2 was used, the bacteriophage did not impact on *B. cereus* on storage with less than 1 log reduction achieved in 24h at 25°C and growth occurring when the mashed potato was stored at 4°C.

Although the work was carried out using *B. cereus*, some of the bacteriophage were shown to be effective against species closely related to *B. cereus*; *B. thurungiesis*, *B. myciodes* and *B. licheniformis*.

Of the 3 genera selected to be representative of the Enteric bacteria, the only paper passing the criteria for the critical review was a study using bacteriophage to control *E. coli* in UHT and raw milk. Data revealed that bacteriophage treatment of *E. coli* delivered a >4 log reduction in UHT milk stored at 25°C and 5-9°C for up to 24h and 168h respectively. In raw milk, the bacteriophage cocktails varied in their ability with one cocktail delivering a >4 log reduction over a 24-168h period and the other allowing growth of *E.coli* by the 168h time point when stored at 5-9°C.

A single study investigating the impact of bacteriophage on the level of lactic acid bacteria met the criteria of the critical review process. The work involved the use of bacteriophage to control lactic acid bacteria in beer (4.3% ethanol v/v) and showed that levels of 10^2 cfu per ml *Lactobacillus brevis* could be reduced to below the limit of detection in 24h. Higher levels of *L. brevis* (10^3 and 10^4 cfu per ml) were lowered beyond the limit of detection in 48h.

Key finding 27: Bacteriophage showed a mixed performance in ability to control spoilage organisms in foods and beverages.

Specificity of bacteriophage

The specificity of the bacteriophage is a key factor in the efficacy of antimicrobial treatments in food products. One of the key known benefits of bacteriophage is the limited host range that enables them to target specific bacterial species (Sillankorva et al. 2012).

Most of the studies included in the review for *L. monocytogenes* evaluated commercially produced bacteriophage based products Listex™ P100 and Listshield™ on a selection of different foods. Both Listex™ P100 and A511 used by (Guenther et al. 2009), are reported to have a broad host range within the genus Listeria (Zink and Loessner, 1992, Hagens and Loessner 2014). Listshield™ (formerly known as LMP:-102) is a 6 strain bacteriophage cocktail that has been reported to be specific *for L. monocytogenes* (Anon 2016). In 2009, Pasternak and Sulakvelidze published a patent describing a cocktail of 6 bacteriophages (named LMP-102), which was tested against 160 isolates of *L. monocytogenes* of which 89% were susceptible to the cocktail. It was also noted by Guenther et al. (2009), that bacteriophage efficacy can be isolate-specific.

The majority of the bacteriophage specificity data for *Salmonella* focussed on the ability of the bacteriophage to infect *Salmonella* isolates rather than non-*Salmonella* isolates. Hungaro et al. (2013) carried out a small assessment of the host range of a cocktail of 5 ψ SE bacteriophages. Data from this work indicated that the cocktail of 5 ψ SE bacteriophages was highly specific, with lysis occurring in 7 out of 7 Salmonella isolates analysed and 1 out of the 7 non-*Salmonella* isolates analysed. Results from the other specificity trails for *Salmonella* revealed that the bacteriophage used were not effective against all of the salmonellae tested. The cocktails used by Heyse et al. (2015) and Jianxiong et al. (2010) had a specificity of 93.8% and 100% respectively.

Very little information on the specificity of *S. aureus* bacteriophages was published in the papers studied in the review. Analysis of a selection of bacteriophages by El Haddad (2013) revealed that bacteriophage Team1 and phage Y lysed 93% and 88% of the 25 *S. aureus* and *S. xylosus* isolates tested. The other 3 bacteriophages analysed in the same study were capable of lysis in 40.4-61.4% of the panel of 57 *S. aureus* and S. *xylosus* strains.

All 3 papers detailing work carried out with *Bacillus* spp. carried out specificity trials. Hakdong et al. (2011) focussed mainly on the susceptibility of *B. cereus* isolates with 6 out of 7 isolates being lysed. An extended host range in the same study showed that the bacteriophage JBP901 was also effective against other members of the *B. cereus* group, but did not cause lysis in other *Bacillus* species (*B. subtilis* and *B. megaterium*) or in 4 non-*Staphylococcus* isolates. Studies carried out by Nadeeka et al. (2014) showed that JBP901 were able to lyse 76.5% food isolates and 87.5 human isolates. No activity was obtained against 50 *B. subtilis* isolates and 12 strains of *B. licheniformis* (Nadeeka et al. 2014). Analysis of the host range of 2 other *Bacillus* bacteriophage FWLBc1 and FWLBc2 indicated that FWLBc2 had a greater host range. FWLBc2 lysed 75% of the 7 *B. cereus* isolates tested compared to FWLBc1 that lysed 50% of the 7 *B. cereus* strains analysed (Lee et al. 2011).

McClean et al. (2011) reported limitations in the host range of the *E. coli* bacteriophages used in their investigations. No specificity data was recorded in the single paper on the impact of bacteriophage in lactic acid bacteria in beer (Deasy et al. 2011).

Bacteriophage specificity was reported in half of the STEC studies included in the review. Analysis of specificity was carried out by looking at the infectivity in a range of STEC isolates. Only 3 papers reported infectivity against more than 20 strains. Two papers also reported on infectivity against other

non-host strains. The majority (9) of the challenge studies were inoculated with only one STEC strain, 2 and 3 strains were used in 1 study each, 5 STEC strains were inoculated into foods that underwent bacteriophage treatment in 3 trials. Host range is important for biocontrol applications as if it is too narrow then the bacteriophage may be ineffective. If however it is non-selective with regards to target species, there could be disruptions to the micro-ecology of the environment, for example, with alterations to gut microflora (Hudson et al. 2013, Tomat et al. 2013). This is especially important when looking at members of the enteric bacteria which make up part of the natural gut microflora.

Key finding 28: Evidence suggests that bacteriophage are specific to their target host, however it is likely that not all strains within a species will be susceptible to a single bacteriophage strain.

Limitations on the use of bacteriophage treatments on foods

The food matrix has an impact on the ability of bacteriophage to reduce the microbial load. Some of the factors associated with a reduction in bacteriophage efficacy discussed in the literature were:

- The influence of intrinsic food factors such as pH, ionic strength and other components such as whey milk protein that impede the binding of bacteriophage to the host cell ligand (Hudson et al. 2005, Gill et al. 2006);
- Non-specific binding of the bacteriophage to food components that hinders binding of the target microorganisms (Anany et al. 2011);
- Changes in food matrices that occur during production, ripening or storage (Guether et al. 2009);
- The availability of liquid to assist the passive diffusion of the bacteriophage which occurs more readily in liquid foods compared to solid food matrices (Guether et al. 2009);
- Uneven and large surface area which physically limits the distribution of bacteriophage (Guether et al. 2009 and Guether et al. 2012);
- Microorganisms embedded or internalised in the food matrix which prevents contact with the bacteriophage (Rossi et al. 2011, Chibeau et al. 2013 and Hyun-Wol et al. 2013).

Key finding 29: The food matrix plays a significant role in the success of bacteriophage treatment of microorganisms in foods. It is important that the application of the bacteriophage is optimised for each food, and takes into consideration the concentration volume and dose as well as the timings of bacteriophage application.

A few studies explored the impact of combining bacteriophage treatments with another antimicrobial treatment. A selection of combination treatments were used with bacteriophage to treat samples contaminated with foodborne pathogens.

In work by Zinno et al. (2014), combination treatments were applied to sprouted seeds, tomato plants and fruit, in the form of bacteriophage treatment in combination with the antagonistic bacterium *Enterobacter ashburiae*. For sprouted seeds, a treatment of MOI log 6 and 10^6 cfu/g *E. ashburiae* over 96 hours, produced a reduction of 5.9–6.8 logs, showing an increased reduction from the bacteriophage treatment alone by 2.5 logs (Jianxiong et al. 2010). Evidence suggests that by co-inoculating antagonistic bacteria with bacteriophage, the target organisms are made more susceptible to the bacteriophage infection. It was postulated that physiological changes in the response of the *Salmonella*, or antimicrobials expressed by the antagonistic bacteria could have enhanced the lytic

activity of the bacteriophage (Jianxiong et al. 2010). The addition of antagonistic bacteria to the tomato plants and fruits however did not improve the susceptibility of *Salmonella* to bacteriophage treatments (Jianxiong et al. 2009). It was thought that the activity of the bacteriophage on growing plants could be affected by the bacteriophage binding to soil particles via electrostatic interactions which would prevent contact with target organism and loss of infectivity. Infectivity could also be affected by exposure to UV radiation.

Bacteriophage treatment in combination with produce washing was also trialled on broccoli, cantaloupe and strawberries. The products were treated with the bacteriophage for 24 hours and then subjected to a levulinic acid produce wash for 1 or 5 minutes. The addition of the bacteriophage treatment increased the reduction in *Salmonella* by over 1 log for the broccoli and cantaloupe products compared to the produce wash only (Magnone et al. 2013).

The use of bacteriophage combination treatments to reduce STEC contamination on fresh produce was also investigated. Studies using bacteriophage in combination with the essential oil extract 0.5% trans-cinnamaldehyde, achieved a 5 log reduction to below the limit of detection after 10 minutes and 1 hour at temperatures equal to and greater than 4°C for spinach and lettuce respectively. The authors suggested that the action of the essential oil on the membrane fatty acid of the STEC could allow the bacteriophage to attach to the membrane more readily allowing for faster infection (Viazis et al. 2011).

Another combination treatment using a bacteriophage treatment followed by a produce wash was trialled on broccoli, cantaloupe and strawberries. The products were treated with the bacteriophage for 24 hours and then subjected to a levulinic acid produce wash for 1 or 5 minutes. The results with co-treatment of bacteriophage treatment and produce wash were the same as the produce wash alone, which was a reduction of 4.1 to 4.6 logs to below the limit of detection, it is therefore not possible to see if the bacteriophage treatment had any additional effect. The trials show a possibility of using a combined treatment of produce wash with bacteriophage treatment to mitigate the risk of potential pathogen growth post treatment. (Magnone et al. 2013)

Four studies investigated the use of combination treatments to reduce the level of *L. monocytogenes* in foods. The key benefit of co-application of bacteriophage with an additional antimicrobial (such as 2.8% potassium lactate, 2.8% potassium lactate-0.2% sodium diacetate and 50 ppm nisin) was the prevention of growth over life compared to the untreated controls (Soni et al. 2012, Chibeu et al. 2013, and Kamlesh et al. 2014). Work by Holck and Berg (2009), revealed that the addition of the antagonistic bacterium *L. sakeii* 1h post addition of bacteriophage, prevented the growth of *L. monocytogenes* in cooked ham stored at 10°C for 28d. Additional results from the same study showed that the bacteriophage/*L. sakeii* combination treatment was able to lower the levels of *L. monocytogenes* in cooked ham (stored at 4°C for 28d) by 1 log.

Research by Tabla et al. (2012) reported a synergistic effect between the bacteriophage treatment and high pressure processing (HPP). A combination of bacteriophage of MOI log 4 and HPP treatments of 400 and 500mPa were capable of lowering the numbers of *S. aureus* present in pasteurised whole milk which was not achieved with the use of the bacteriophage alone.

Key finding 30: In general the use of bacteriophage treatment in combination with another antimicrobial treatment delivered a greater log reduction than the bacteriophage treatment alone.

Nearly all of the bacteriophage used in the *S. aureus* studies were lytic bacteriophage with only 1 out of the 5 papers using lysogenic bacteriophage (Garcia et al. 2009). Interestingly, the lysogenic bacteriophage did not control the levels of *S. aureus* in milk and during storage at 37°C, the numbers of the spiked host increased over time. The results suggest that lytic bacteriophage deliver a more effective reduction in microbial numbers compared to lysogenic strains.

Key finding 31: Evidence indicates lytic bacteriophages that target *S. aureus* are more effective in reducing microbial load in foods than lysogenic bacteriophage.

Another observation by the review team was that on some occasions the storage conditions, i.e. the storage temperature used, was not always appropriate for the food product. An example of this was milk stored at 37°C (Obeso et al. 2010), which would be typically stored at 2-8°C over product life. It is known that bacteriophage replication is optimal when the bacterial cells are in mid-log phase therefore when organisms are stored under sub-optimal growth conditions; the temperature is likely to impact on bacteriophage efficacy. Storage temperature is thought to be one of the main factors that influence the ability of bacteriophage to control microbial contamination along with contact time and the MOI (Viazis et al. 2011).

Key finding 32: Bacteriophage performance is influenced by contact time and temperature

The emergence of bacteriophage resistant organisms

The development of bacteriophage resistance is an important point for consideration due to its potential to significantly impact the efficacy of bacteriophage treatments. Analysis of the evidence tables showed that some of the studies on *L. monocytogenes*, *Salmonella* spp and *S. aureus* included a section on bacteriophage resistance. Data obtained shows that bacteriophage resistance has been observed in some of the work included in this review. The potential for bacteriophage resistance to occur in *L. monocytogenes* isolates was reported in 2 out of the 15 papers used in the review (Rodriguez-Rubio et al. 2015, Holck and Berg 2009). Additional research indicates that some *L. monocytogenes* are naturally bacteriophage resistant (Carlton et al. 2005).

In order to reduce the likelihood of bacteriophage resistance there are 4 measures that should be adhered to:

- Use of bacteriophage with broad host range;
- Application of bacteriophage with different host ranges in cocktails preferably in rotating application schemes;
- Treatment prior to packaging and shipment to prevent re-entry of bacteriophage resistant flora into factory; and
- Avoidance of incorporating old product into new batches i.e. old/young smearing in cheese production (Guenther et al. 2009 and Guenther et al. 2012).

Interestingly, the use of combination treatments (bacteriophage and bacteriocin reduced the level of bacteriophage resistance that occurred in *L monocytogenes* spiked into milk products (Rodriguez-Rubio et al. 2015).

Key finding 33: Natural bacteriophage resistance can occur in *L. monocytogenes*.

Key finding 34: Strategies can be put in place to reduce the potential for bacteriophage resistant populations occurring.

The use of a bacteriophage cocktail to reduce the load of *Salmonella* on a range of foods enabled the bacteriophage sensitivity to be maintained up to 6 days post treatment (Hungaro et al. 2013 and Guenther et al. 2012). One of the key reasons suggested for this was the short contact time used for the bacteriophage cocktail. In addition, research by Zinno et al. (2014) found that remaining *Salmonella* isolates were sensitive to the P22 bacteriophage.

The potential for the development of bacteriophage resistance was discussed in 1 out of the 5 papers included in the review for *S. aureus*. Although no specific research was carried out Tabla et al. (2012) quoted an emergence rate of bacteriophage insensitive mutants of approximately $1x10^{-6}$ in S. aureus SA9 (following treatment with a mixture of bacteriophages philPLA88 and philPLA35 at a MOI of log 2).

Key finding 35: Bacteriophage resistance was not reported in *Salmonella* spp. or *S. aureus* however few papers addressed the issue of bacteriophage resistant mutants.

An investigation was not carried out on the development of bacteriophage resistance in 3 of the organisms included in this critical review (lactic acid bacteria, *Bacillus* spp, and the enteric bacterium *E. coli*).

Key finding 36: Limited information is available on the formation of bacteriophage resistant mutants in spoilage organisms.

Protection against product recontamination

The main indicator of the ability of bacteriophage to protect food against microbial recontamination is the presence of residual bacteriophage activity. There are 2 components of residual activity persistence of the bacteriophage on the food and the maintenance of bacteriophage infectivity. Of the papers included in the review several determined the stability of the bacteriophage on the food during the storage period. Data suggested that some of the bacteriophage applied were capable of persisting on the food surface (Chibeau et al. 2013, Soni et al. 2012, Rossi et al. 2011, Soni and Ramakrishna 2010, Tranuk et al. 2014, Viazis et al. 2011). The level of bacteriophage present on the food would have been determined by a plaque assay, which would give an indication of the infectivity of the remaining bacteriophage. Although the stability of the bacteriophage on food has been established for a selection of bacteriophage, no studies have been carried out on whether they retain residual activity on re-challenge with the target organism.

Key finding 37: Residual activity was not investigated in the reported studies, although some of the research determined the stability of the bacteriophage over the storage period.

Safety issues on the use of bacteriophage in foods quoted in the literature

Some safety points were raised in section 2 of the report and the key findings are listed under key finding 38 and 39.

Key finding 38: Although bacteriophage are being approved for use in the food industry one of the main barriers to their wider use is the formulation of guidelines for their safe and effective use. The three key safety concerns are; toxicological safety, emergence of resistance to biocide and antimicrobials linked to the use of bacteriophage, and the risk of release of bacteriophage into the environment.

Key finding 39: A peer reviewed expert consensus on the quality and safety requirements is available for medicinal products which can be modified for use in the food industry.

Some of the papers in the review highlighted some important considerations for specific food pathogens. One consideration raised with *S. aureus* was the potential of the stress caused by bacteriophage infection to trigger the expression of the enterotoxin genes. The impact of bacteriophage on toxin levels was investigated in a single study used in the critical review. Results from the research suggested that *Staphyloccus* entertoxin type C (SEC) was not produced in the presence of bacteriophage during maturation. Further research would be required with different isolates capable of producing a range of toxin types before firm conclusions could be drawn on the safety of bacteriophage with respect to Staphylococcal enterotoxin.

Key finding 40: Consideration should be given to the impact of bacteriophage infection on *Staphylococcus* enterotoxin production in *S. aureus* isolates.

Furthermore, bacteriophage specificity should be taken into account when assessing the safety of bacteriophage treatments. If the host range is broad and is non selective with regards to the target species, then this may impact on the micro-ecology of the environment. For example, with alterations to gut microflora (Hudson et al. 2013, Tomat et al. 2013) this is especially important when looking at members of the enteric bacteria which make up part of the natural gut microflora.

Key finding 41: The specificity of bacteriophage plays an important role in determining efficacy against organisms of interest such as foodborne pathogens. The potential impact bacteriophage treatments may have on the microflora of the human intestine should be taken into account, especially for bacteriophage targeting pathogenic enteric bacteria.

It is important to be aware of the presence of virulence factors and encoded genes in bacteriophages to be included in food products for biocontrol purposes, to prevent modification of bacterial phenotype by bacteriophage gene expression (Tomat et al. 2013). Only bacteriophages without these genes should be considered for use in the treatment of food. (Young-Duck et al 2013).

Key finding 42: Checks must be carried out to ensure that the bacteriophages to be used for food applications do not contain toxin genes or virulence factors. This is particularly important for STEC bacteriophage some of which are known to carry genes responsible for shiga toxin formation and other virulence factors.

5 GAPS IN CURRENT KNOWLEDGE

The critical review highlighted several gaps in knowledge listed below:

Bacteriophages

Key finding 7: Scarcity of data available for the use of bacteriophage to control spoilage organisms on foods compared to foodborne pathogens. Research has focussed on key food borne pathogens; *L. monocytogenes*, *Salmonella* spp. STEC and *S. aureus*. *Campylobacter* spp. was not included in the review as the studies on this pathogen investigated its control in bioflms and live animals which were outside the scope of the current review.

Key finding 9: No information was available on the use of bacteriophage to control natural microbial contamination present on food samples.

Key finding 10: The addition of bacteriophage in most of the studies is carried following the spike of bacteria followed by a period of 'acclimatisation' on the food matrix.

Key finding 37: Residual activity was not investigated in the reported studies, although some of the research determined the stability of the bacteriophage over the storage period.

Key finding 35: Bacteriophage resistance was not reported in *Salmonella* spp. or *S. aureus* however few papers addressed the issue of bacteriophage resistant mutants.

Key finding 36: Limited information is available on the formation of bacteriophage resistant mutants in spoilage organisms.

Bacteriophage-encoded enzymes

Although not included in the critical review the review team felt that the use of enzymes encoded by bacteriophage which can be used as an antimicrobial would be an area of interest to the food industry. Bacteriophage produce enzymes that break down the peptidoglycan within the cell wall to release the bacteriophage particles at the end of their lifecycle, and are known as endolysins (O' Flaherty et al. 2009). Endolysins have been successfully used to eliminate Gram positive organisms including *Streptococcus*, *Staphylococcus* and *Bacillus* (Fishetti 2010).

Evidence indicates that endolysins may also be capable of killing some Gram negative organisms and these have been termed 'enzobiotics' (Nelson et al. 2001). Endolysin activity against Gram negative microorganisms is usually constrained by the protective outer membrane restricting the abaility of the bacteriophage to reach the peptidoglycan (Nikaido 2003). However reagents such as EDTA can be used to 'permeablize' the outer membrane (Mastromatteo et al. 2010, Briers et al. 2011 and Walmagh et al. 2011). Recent studies have reported that a *Salmonella* phage endolysin (Lys68) is capable of killing Gram negative bacteria in the presence of weak acids (Oliveira et al. 2014). The author proposed that a potential application of Lys68 would be as a food preservative against *Ps. fluorescens*. A genetic screen has also been developed for identifying lysins that could be effective against Gram negative organisms (Schuh et al. 2009).

Further research however, is needed to elucidate the endolysin dosage required for effective antimicrobial activity for both Gram positive and Gram negative organisms (O' Flaherty et al. 2009). Other aspects of the potential use of endolysins such as cost, scalability, safety and stability in food matrices also remain to be determined.

6. CONCLUSIONS AND RECOMMENDATIONS

This review focused on 2 key objectives.

Objective 1: Confirm current situation on the use of bacteriophage as biocontrol agents in food

A literature review was carried out to establish the published information base on the current global use of bacteriophage as biocontrol agents in food. Full details of the results of the review are outlined in section 2. The first objective covered 3 key areas:

- Current global use of bacteriophage within the food industry to control microorganisms on food products;
- Published controls for the use of bacteriophage within the food industry;
- Safety considerations and recommendations on the use of bacteriophage as biocontrol agents on foods.

Key finding 1: Generally Regarded As Safe (GRAS) approved, commercially produced bacteriophage are being used as food additives in the US and as processing aids in parts of Europe. The products are being used to reduce the pathogen load of *L. monocytogenes*, *Salmonella enterica* and *E. coli* O157:H7 on a range of named food types; poultry, red meat, RTE foods, fish, shellfish as well as processed fruits and vegetables.

Key finding 2: The major use of commercially produced bacteriophage to date is in the pre-harvest control of foodborne pathogens including STEC and *Salmonella*.

Key finding 3: Although bacteriophage is being approved for use in the food industry, one of the main barriers to their wider use is the formulation of guidelines for their safe and effective use. The three key safety concerns are: toxicological safety; emergence of resistance to biocide and antimicrobials linked to the use of bacteriophage; and the risk of release of bacteriophage into the environment.

Key finding 4: Research on the use of bacteriophage in the clinical sector is available, however official guidelines/regulations are yet to be published for bacteriophage therapeutic products in the US or Europe.

Key finding 5: A peer reviewed expert consensus on the quality and safety requirements is available for therapeutic products which could be modified for use in the food industry.

Key finding 6: At present there are no specific controls in place that are specific to the use of bacteriophage treatments on foods. There are however several regulations that must be complied with when using bacteriophage treatments in/on food products across Europe:

i) Hygiene regulation (EC) No 853/2004 applicable to bacteriophage treatments that are to be applied to the surface of meat or fish to remove bacterial contamination;
ii) Food Additive Regulation (EC) No 1333/2008 detailing controls specific to food additives and food processing aids; and
iii) General food law which states that food must be safe for human consumption

Conclusion 1: Although commercially available bacteriophage products are used to control microbial levels in the global food chain, further work is needed to establish and agree common guidelines for their safe and effective use. In some cases however bacteriophage treatments will require approval before they can be used on/in foods.

Conclusion 2: Knowledge can be shared with the pharmaceutical sector, which has expertise in the use of bacteriophage to control microbial populations. Documents produced by colleagues in the field of medical bacteriophage therapy can be adapted to fit food industry requirements.

Objective 2: Critical review to assess the potential for the use of bacteriophage as biocontrol agents on foods.

Objective 2 addressed 3 key points listed in the proposal:

- Provide a list of conclusions on the efficacy of bacteriophage as biocontrol agents on food products including: limitations to the use of bacteriophage as biocontrol agents on food products and the safety of bacteriophage as biocontrol agents on food products;
- Give recommendations on the efficacy and safety of bacteriophage as biocontrol agents on food products;
- Highlight gaps in knowledge that were identified during the review and provide recommendations for further research to fill these gaps.

Following the critical review, a number of key findings were noted by the review team. The findings are spilt into 3 sections to address the points listed in the initial proposal namely: efficacy of bacteriophage treatments; safety issues associated with the use of bacteriophages in foods quoted in the literature; and gaps in knowledge identified during the review.

Efficacy of bacteriophage treatments as biocontrol agents on food products

The key findings in this section are listed under the criteria used to assess the potential of bacteriophage as effective biocontrol agents in foods:

- Compatibility of food products and bacteriophage treatments;
- Specificity of bacteriophage;
- Limitations on the use of bacteriophage as biocontrol agents on foods;
- Possible issues arising from the use of bacteriophage as biocontrol agents on foods, e.g. the emergence of bacteriophage resistant organisms;
- The ability of bacteriophage to protect against food product recontamination.

Foods compatible with bacteriophage treatments

Conclusions were drawn on the key findings for each of the individual pathogens and collectively for the spoilage organisms included in the review**.**

L. monocytogenes

Key finding 13: Bacteriophage is capable of delivering up to a 2.5 log reduction in *L. monocytogenes* on cooked meat. The activity of the bacteriophage has been shown to be enhanced when meat is packed using MAP or vacuum packing.

Key finding 14: The application of bacteriophage can deliver a 2 log reduction in *L. monocytogenes* on processed meat.

Key finding 15: Bacteriophage did not effectively control the level of *L. monocytogenes* on some fruit; however they were successful in lowering the numbers of *L. monocytogenes* on salad vegetables and vegetables by 1-2 log.

Key finding 16: Data suggests that bacteriophage are more effective at reducing *L. monocytogenes* in soft cheese compared to hard cheese, although high levels of bacteriophage are required to control numbers over long term storage.

Key finding 17: Bacteriophage at a level of MOI log 4 were able to lower the level of spiked *L. monocytogenes* by up to 3 logs in fish and seafood.

Key finding 18: Bacteriophage have been shown to effectively reduce the level of *L. monocytogenes* in fruit juice by up to 4 log and in milk by 4 logs after 48h and 6 log after 96h.

Conclusion 3: The published literature indicates that there is a potential for bacteriophage to be used to control the level of *L. monocytogenes* on several food types; cooked and processed meats, salad vegetables, vegetables as well as fish and seafood

Salmonella

Key finding 19 : Bacteriophage are capable of reducing *Salmonella* contamination on raw meat by up to 3 logs and were shown to be equivalent to alternative treatments including lactic and peroxyacetic acid.

Key finding 20: Bacteriophage treatments were able to effectively reduce *Salmonella* contamination on a selection of fruit and vegetables by up to 6.8 logs with the exception of strawberries and tomatoes.

Key finding 21: Bacteriophage treatment of milk and soft drink products with an MOI of log 8 resulted in a reduction in *Salmonella* of up to 4.5 log.

Conclusion 4: Evidence suggests that bacteriophage treatments could be capable of delivering effective reductions in numbers of *Salmonella* spp on raw meat, some fruit and vegetables as well as in milk and soft drinks.

Key finding 22: Bacteriophage treatments are able to reduce the level of STEC on meat by up to 4 log. Evidence suggests that intervention directly post slaughter may provide the greatest efficacy of bacteriophage inactivation of STEC.

Key finding 23: Studies indicate that bacteriophages are capable of reducing the level of STEC on fresh produce by up to 4 logs on vegetables, 2.97 logs on fruit and 3 logs on salad leaves. The mode of application of bacteriophage treatment may also be important, with spray treatments reported to be more effective than immersion treatments.

Key finding 24: Limited data is available on bacteriophage treatments of STEC in liquids. However, available data suggest that MOI of log 4 can deliver at least a 1 log reduction in STEC.

Conclusion 5: The literature suggests that bacteriophage treatments may be effective for the reduction of STEC in meat products and fresh produce. Optimisation of the mode of treatment application, bacteriophage concentration used as well as contact time and temperature is required to ensure that the bacteriophage treatment is fit for purpose.

Staphylococcus aureus

Key finding 25: Bacteriophage are capable of reducing the level of *S. aureus* in cheese by up to 5 logs during storage for 24-336h.

Key finding 26: The ability of bacteriophage to reduce *S. aureus* in milk is dependent on the type and is influenced by storage temperature and MOI.

Conclusion 6: Although bacteriophage shows potential to control *S. aureus* in dairy products, more research is needed to confirm their suitability for use.

Key finding 27: Bacteriophage showed a mixed performance in ability to control spoilage organisms in foods and beverages.

Specificity of bacteriophage

Key finding 28: Evidence suggests that bacteriophage are specific to their target host, however it is likely that not all strains within a species will be susceptible to a single bacteriophage strain.

Conclusion 7: It is unlikely that a single bacteriophage will be effective against all strains of a particular bacterial species, therefore the use of a cocktail of bacteriophage is recommended to reduce the pathogen/spoilage load on food.

Limitations on the use of bacteriophage on foods

Key finding 29: The food matrix plays a significant role in the success of bacteriophage treatment of microorganisms in foods. It is important that the application of the bacteriophage is optimised for each food, and takes into consideration the concentration volume and dose as well as the timings of bacteriophage application.

Key finding 30: In general the use of bacteriophage treatment in combination with another antimicrobial treatment delivered a greater log reduction than the bacteriophage treatment alone.

Key finding 31 Evidence indicates lytic bacteriophages that target *S. aureus* are more effective in reducing microbial load in foods than lysogenic bacteriophage.

Key finding 32: Bacteriophage performance is influenced by contact time and temperature.

Conclusion 8: The use of bacteriophage to control populations of pathogens or spoilage organisms is influenced by the food itself as well as the storage environment. All bacteriophage treatments should be optimised and validated to ensure that they are fit for purpose.

Conclusion 9: Data from this review indicate that bacteriophage treatments are not a 'magic bullet' and that they should be used in the hurdle approach to food safety.

Conclusion 10: It is recommended that lytic bacteriophage should be used in preference to lysogenic bacteriophage as results suggest that lytic bacteriophage are more effective than lysogenic bacteriophage.

Possible issues arising from the use of bacteriophage, e.g. the emergence of bacteriophage-resistant organisms

Key finding 33: Natural bacteriophage resistance can occur in *L. monocytogenes*.

Key finding 34: Strategies can be put in place to reduce the potential for bacteriophage resistant populations occurring.

Key finding 35: Bacteriophage resistance was not reported in *Salmonella* spp or *S. aureus*, however few papers addressed the issue of bacteriophage induced mutations in the microbial population.

Key finding 36: Limited information is available on the formation of bacteriophage resistant mutant isolates in spoilage organisms.

Conclusion 11: There is insufficient published data to assess the full impact of microbial resistance to becteriphage on the efficacy of bacteriophage to reduce microbial contamination. Therefore more research is required. It is also recommended that strategies to reduce the potential for formation of bacteriophage resistant mutants are implemented when bacteriaophage treatments are developed.

The ability of bacteriophage to protect against product recontamination

Key finding 37: Residual activity was not investigated in the reported studies, although some of the research determined the stability of the bacteriophage over the storage period.

Conclusion 12: From the evidence to date, the suggested use of bacteriophage would be as a processing aid to reduce the level of microorganisms at a set time point. Further studies are needed to determine the residual activity of bacteriophage on food and their ability to prevent recontamination of food products. In addition, research on the stability of bacteriophage in food relevant conditions (e.g. pH and water activity) is also recommended.

Safety issues associated with the use of bacteriophage in foods quoted in the literature

Key finding 38: Although bacteriophage are being approved for use in the food industry one of the main barriers to their wider use is the formulation of guidelines for their safe and effective use. The three key safety concerns are: toxicological safety; emergence of resistance to biocide and antimicrobials linked to the use of bacteriophage; and the risk of bacteriophage release into the environment.

Key finding 39: A peer reviewed expert consensus on the quality and safety requirements is available for medicinal products which can be modified for use in the food industry.

Key finding 40: Consideration should be given to the impact of bacteriophage infection on *Staphylococcus* enterotoxin production in *S. aureus* isolates.

Key finding 41: The specificity of bacteriophage plays an important role in determining efficacy against organisms of interest such as foodborne pathogens. It is important to take into account the potential impact bacteriophage treatments may have on the microflora in the human intestine, especially for bacteriophage targeting members of the enteric bacteria.

Key finding 42: Checks must be carried out to ensure that the bacteriophages to be used for food applications do not contain toxin genes or virulence factors. This is particularly important for STEC bacteriophage some of which are known to carry genes responsible for shiga toxin formation and other virulence factors.

Conclusion 13: The literature details methods to ensure safety of bacteriophage for use in the food supply chain, however further work is needed to create common guidelines for safe manufacture and effective application of bacteriophage treatments in the food industry. Amongst the issues to be addressed are i) the impact of bacteriophage infection on toxin formation in known toxin forming bacteria i.e. *S. aureus* and members of *Bacillus* spp. and *Clostridium* spp. ii) the effect of bacteriophage on microflora in the human intestine and in waste effluent from food processing plants and iii) verification that bacteriophage to be used in foods/ food processes do not contain toxin genes or other virulence factors.

Highlight gaps in current knowledge

The critical review highlighted several gaps in knowledge listed below:

Key finding 7: Scarcity of data available for the use of bacteriophage to control spoilage organisms on foods compared to foodborne pathogens. Research has focussed on key food borne pathogens; *L. monocytogenes*, *Salmonella* spp. STEC and *S. aureus*. *Campylobacter* spp. was not included in the review as the studies on this pathogen investigated its control in bioflms and live animals which were outside the scope of the current review.

Key finding 9: No information was available on the use of bacteriophage to control natural microbial contamination present on food samples.

Key finding 10: The addition of bacteriophage in most of the studies is carried following the spike of bacteria followed by a period of ‘acclimatisation’ on the food matrix.

Conclusion 14: More research is required to establish the potential of bacteriophage to control spoilage organisms in foods. To ensure that data can be compared between studies it is recommended that the investigations contain the following details:

Study characteristics	Requirements
Use of appropriate organisms for food type used	All strains appropriate to food type
Method of inoculation of microorganism into food samples available	Fully available including level of challenge inoculum
The number of organisms used, their source – culture collection or food isolates	More than 3 isolates used. Mixture of culture collection and food isolates
Method of phage application to the food available	Fully available including phage dose
Number of time points tested	Three or more
Number of samples/replicates used	Triplicate samples of one batch or single samples done in triplicate at each time point
Inclusion of relevant controls	Use of appropriate study controls i.e. positive and negative samples for each sample type
Statistical analysis performed on the raw data	Full analysis carried out
Analysis of data performed – log reduction calculated	Log reductions provided

Conclusion 15: Further work is needed to establish the performance of bacteriophage to control microbial contamination in situations that would commonly occur in the food industry. This should include:

iii) The use of naturally contaminated foods in pilot trials and

iv) An investigation on whether bacteriophage treatments are effective against microorganisms introduced onto the food after the application of bacteriophage into/onto the food

Although not included in the critical review the review team felt that the use of enzymes encoded by bacteriophage which can be used as an antimicrobial would be an area of interest to the food industry. Bacteriophages produce enzymes that break down the peptidoglycan within the cell wall to release the bacteriophage particles at the end of their lifecycle, and are known as endolysins (O' Flaherty et al. 2009). Endolysins have been successfully used to eliminate Gram positive organisms including *Streptococcus*, *Staphylococcus* and *Bacillus* (Vishetti 2010).

Evidence indicates that endolysins may also be capable of killing some Gram negative organisms and these have been termed 'enzobiotics' (Nelson et al. 2001). Endolysin activity against Gram negative microorganisms is usually constrained by the protective outer membrane to reach the peptidoglycan (Nikaido 2003), however reagents such as EDTA can be used to 'permeablize' the outer membrane (Mastromatteo et al. 2010, Briers et al. 2011 and Walmagh et al. 2011). Recent studies have reported that a *Salmonella* phage endolysin (Lys68) is capable of killing Gram negative bacteria in the presence of weak acids (Oliveira et al. 2014). The author proposed that a potential application of Lys68 would be as a food preservative against *Ps. fluorescens*. A genetic screen has also been developed for identifying lysins that could be effective against Gram negative organisms (Schuh et al. 2009).

Further research however is needed to elucidate the endolysin dosage required for effective antimicrobial activity for both Gram positive and Gram negative organisms (O' Flaherty et al. 2009). Other aspects of the potential use of endolysins such as cost, scalability, safety and stability in food matrices also remain to be determined.

Conclusion 16: The use of bacteriophage lytic enzymes is a promising approach to the control of pathogens and spoilage organisms in foods. One of the key benefits of lytic enzymes is the lack of reliance on virus replication to achieve lysis of the cell. More research is required to determine their fitness for purpose in foods.

7. LIMITATIONS OF REVIEW

There were several limitations to the review process which are listed below:

- STEC bacteriophage work was focused on *E. coli* O157 with very little data available for the other serotypes that have been isolated from raw beef or sprouted seeds.
- A paucity of information on the control of spoilage organisms by bacteriophage treatments compared to the 5 selected food borne pathogens.
- None of the studies had evaluated the use of phage in the field either in pilot facilities or in the factory environment.
- Of the 105 papers chosen for use in the evidence gathering data, many were not relevant despite fulfilling the search criteria. Reasons for exclusion were, for example:-
    - A lack of data required in the evidence tables e.g. contact time and temperature and dose of bacteriophage.
    - Studies focussed on the characterisation of bacteriophage rather than its ability to reduce microorganism on foods.
    - Obtaining a score below the cut off threshold applied.
- Despite the above limitations, some useful literature evidence was gathered on the use of bacteriophage to control the level of selected pathogens and spoilage organisms. The key findings noted by the team have enabled the production of useful and informative set of conclusions and recommendations.

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APPENDIX 1

Scoring sheet used for the critical review

criteria	Score of 2	Score of 1	Score of 0
Method of phage propagation and purification available	Fully available	Some details available	No details provided
Characterisation data available for the phage strains used	Fully available	Some details available	No details provided
Use of appropriate organisms for food type used	All strains appropriate to food type	Most strains appropriate to food type	Some strains appropriate to food type
Method of inoculation of microorganism into food samples available	Fully available	Some details available	No details provided
The number of organisms used, their source – culture collection or food isolates	More than 3 isolates used. Mixture of culture collection and food isolates	Two or more isolates used. Mixture of culture collection and food isolates	One strain used. Culture collection or food isolate used
Method of phage application to the food available	Fully available	Some details available	No details provided
Calculation of phage dose available	Fully available	Some details available	No details provided
Method of phage enumeration available	Fully available	Some details available	No details provided
Number of time points tested	Three or more	Two	Less than two
Number of samples/replicates used	Triplicate samples of one batch or single samples done in triplicate at each time point	Duplicate samples of one batch or single samples done in duplicate at each time point	Single samples used at each time point
Inclusion of relevant controls	Use of appropriate study controls i.e. positive and negative samples for each sample type	N/A	No study controls analysed
Statistical analysis performed on the raw data	Full analysis carried out	Some analysis done	No details provided
Analysis of data performed – log reduction calculated	Log reductions provided	Data available for log reductions to be calculated	N/A
Conclusions on the efficacy of the phage/suggested use of the phage	Fully available	Some details available	No details provided
Comparison with other published data	Comprehensive comparison carried out	Some reference to published literature	N/A

APPENDIX 2

Scores for the literature in the critical review

***Bacillus* spp**

Reference	Total Score	%
BA14	**49**	82
BA35	**49**	82
BA37	**45**	75
BA30	**44**	73
BA5	**17**	28
BA36	**12**	20
BA34	**11**	18
BA45	**11**	18
BA9	**9**	15
BA47	***20***	33
BA52	***8***	13
BA2	***5***	8
BA15	***5***	8
BA27	***5***	8
BA28	***2***	8
BA1	***0***	0
BA4	***0***	0
BA6	***0***	0
BA8	***0***	0
BA11	***0***	0
BA13	***0***	0
BA16	***0***	0
BA21	***0***	0
BA25	***0***	0
BA29	***0***	0
BA31	***0***	0
BA33	***0***	0
BA39	***0***	0
BA41	***0***	0
BA44	***0***	0
BA46	***0***	0
BA51	***0***	0
BA53	***0***	0

Reference	Total Score	%
BA55	***0***	0
BA56	***0***	0
BA58	***0***	0
BA59	***0***	0
BA60	***0***	0

***Brocothrix* spp.**

Reference	Total score	%
BR2	**15**	25
BR1	*0*	*0*

Campylobacter spp.

Reference	Total Score	%
CA5	**35**	58
CA9	**35**	58
CA10	**33**	55
CA31	**32**	53
CA18	**28**	47
CA32	**26**	43
CA26	***18***	30
CA4	***16***	27
CA16	***16***	27
CA12	***14***	23
CA2	***11***	18
CA6	***9***	15
CA35	***9***	15
CA20	***7***	12
CA13	***4***	7
CA21	***4***	7
CA8	***2***	3
CA14	***2***	3
CA15	***2***	3
CA24	***2***	3
CA25	***2***	3
CA34	***1***	2
CA22	***0***	0

Reference	Total Score	%
CA27	***0***	0
CA29	***0***	0
CA30	***0***	0

***Citrobacter* spp**

Reference	Total score	%
CI1	**17**	28
CI3	***2***	*3*
CI5	*0*	*0*

***Clostridium* spp**

Reference	Total score	%
CL114	**29**	48
CL11	**16**	27
CL13	**14**	23
CL115	**14**	23
CL10	**10**	17
CL1	***8***	13
CL5	***8***	13
CL6	***8***	13
CL24	***7***	12
CL113	***7***	12
CL4	***6***	10
CL2	***4***	7
CL3	***0***	0
CL7	***0***	0
CL12	***0***	0
CL16	***0***	0
CL17	***0***	0
CL20	***0***	0
CL21	***0***	0

Escherichia coli

Reference	Total score	%
ES83	**44**	73
ES190	**43**	72
ES179	**41**	68
ES181	**21**	35
ES84	**15**	25
ES25	**12**	20
ES81	**10**	17
ES263	***26***	43
ES153	***19***	32
ES5	***18***	30
ES143	***17***	28
ES201	***11***	18
ES206	***11***	18
ES346	***9***	15
ES104	***6***	10
ES139	***3***	5
ES118	***2***	3
ES2	***1***	2
ES45	***1***	2
ES48	***0***	0
ES56	***0***	0
ES85	***0***	0
ES170	***0***	0
ES191	***0***	0
ES219	***0***	0
ES242	***0***	0
ES268	***0***	0
ES347	***0***	0

***Hafnia* spp**

reference	**Total score**	%
HA1	**16**	27
HA2	***0***	*0*
HA3	***0***	*0*
HA4	***0***	*0*

Lactic acid bacteria

Reference	**Total score**	**%**
LA45	**39**	65
LA25	**16**	27
LA41	**14**	23
LA5	**13**	22
LA173	**5**	8
LA51	**4**	7
LA19	**2**	3
LA48	**1**	2
LA1	***0***	0
LA2	***0***	0
LA4	***0***	0
LA7	***0***	0
LA13	***0***	0
LA14	***0***	0
LA16	***0***	0
LA18	***0***	0
LA20	***0***	0
LA27	***0***	0
LA28	***0***	0
LA30	***0***	0
LA31	***0***	0
LA32	***0***	0
LA33	***0***	0
LA34	***0***	0
LA36	***0***	0
LA38	***0***	0
LA40	***0***	0
LA42	***0***	0
LA43	***0***	0

Reference	Total score	%
LA49	***0***	0
LA52	***0***	0
LA53	***0***	0
LA172	***0***	0

Listeria monocytogenes

Reference	Total score	%
LI73	**50**	83
LI11	**46**	77
LI21	**46**	77
LI119	**46**	77
LI30	**45**	75
LI58	**45**	75
LI84	**45**	75
LI96	**45**	75
LI117	**45**	75
LI74	**43**	72
LI90	**41**	63
LI75	**38**	63
LI106	**38**	63
LI1	**33**	55
LI27	**30**	50
LI97	**30**	50
LI98	**30**	50
LI51	**28**	47
LI14	**24**	40
LI109	**23**	38
LI116	**19**	32
LI8	***34***	57
LI66	***33***	55
LI91	***25***	42
LI57	***24***	40
LI37	***21***	35
LI5	***19***	32
LI31	***16***	27
LI47	***16***	27
LI42	***15***	25
LI56	***15***	25

Reference	Total score	%
LI80	***14***	23
LI16	***11***	18
LI118	***11***	18
LI12	***8***	13
LI43	***5***	8
LI81	***5***	8
LI101	***4***	7
LI64	***2***	3
LI6	***0***	0
LI13	***0***	0
LI15	***0***	0
LI17	***0***	0
LI18	***0***	0
LI19	***0***	0
LI22	***0***	0
LI23	***0***	0
LI26	***0***	0
LI28	***0***	0
LI32	***0***	0
LI35	***0***	0
LI38	***0***	0
LI39	***0***	0
LI40	***0***	0
LI41	***0***	0
LI46	***0***	0
LI48	***0***	0
LI49	***0***	0
LI50	***0***	0
LI52	***0***	0
LI54	***0***	0
LI59	***0***	0
LI63	***0***	0
LI65	***0***	0
LI67	***0***	0
LI68	***0***	0
LI70	***0***	0
LI71	***0***	0
LI72	***0***	0
LI76	***0***	0
LI77	***0***	0

Reference	Total score	%
LI78	***0***	0
LI79	***0***	0
LI82	***0***	0
LI83	***0***	0
LI87	***0***	0
LI88	***0***	0
LI89	***0***	0
LI92	***0***	0
LI93	***0***	0
LI95	***0***	0
LI102	***0***	0
LI103	***0***	0
LI104	***0***	0
LI110	***0***	0
LI114	***0***	0

***Pseudomonas* spp**.

Reference	Total score	%
PS22	**20**	33
PS11	**17**	28
PS7	**14**	23
PS15	**14**	23
PS32	***11***	18
PS28	***6***	10
PS33	***3***	5
PS6	***0***	0
PS8	***0***	0
PS10	***0***	0
PS13	***0***	0
PS14	***0***	0
PS18	***0***	0
PS20	***0***	0
PS25	***0***	0
PS26	***0***	0
PS27	***0***	0
PS31	***0***	0

***Salmonella* spp.**

Reference	Total score	%
SA22	**54**	90
SA88	**54**	90
SA224	**53**	90
SA182	**50**	83
SA257	**50**	83
SA39	**49**	82
SA97	**49**	82
SA129	**49**	82
SA150	**49**	82
SA196	**49**	82
SA12	**46**	77
SA260	**46**	77
SA278	**43**	72
SA132	**39**	65
SA269	**39**	65
SA33	**38**	63
SA73	**38**	63
SA84	**38**	63
SA281	**34**	57
SA79	***43***	72
SA53	***41***	68
SA60	***33***	55
SA282	***33***	55
SA92	***29***	48
SA28	***28***	47
SA105	***28***	47
SA166	***27***	45
SA20	***25***	42
SA45	***24***	40
SA56	***24***	40
SA155	***24***	40
SA280	***22***	37
SA283	***22***	37
SA78	***20***	33
SA4	***18***	30
SA66	***18***	30

Reference	Total score	%
SA164	***18***	30
SA212	***18***	30
SA119	***17***	28
SA211	***16***	27
SA218	***16***	27
SA6	***14***	23
SA197	***14***	23
SA201	***13***	22
SA21	***12***	20
SA51	***12***	20
SA284	***9***	15
SA120	***8***	13
SA237	***8***	13
SA40	***7***	12
SA147	***7***	12
SA54	***6***	10
SA140	***6***	10
SA270	***6***	10
SA82	***4***	7
SA121	***4***	7
SA123	***4***	7
SA143	***4***	7
SA26	***2***	3
SA159	***2***	3
SA2	***0***	0
SA3	***0***	0
SA17	***0***	0
SA38	***0***	0
SA44	***0***	0
SA59	***0***	0
SA96	***0***	0
SA128	***0***	0
SA156	***0***	0
SA165	***0***	0
SA233	***0***	0
SA252	***0***	0
SA259	***0***	0
SA273	***0***	0
SA275	***0***	0
SA276	***0***	0

Reference	Total score	%
SA277	***0***	0
SA279	***0***	0

Staphylococcus aureus

Reference	Total score	%
ST51	**56**	93
ST22	**47**	78
ST76	**47**	78
ST67	**46**	77
ST33	**45**	75
ST35	**44**	73
ST11	**29**	48
ST46	**17**	28
ST13	**7**	12
ST26	**7**	12
ST73	**7**	12
ST32	**6**	10
ST64	**6**	10
ST25	**5**	8
ST45	***7***	12
ST29	***4***	7
ST43	***4***	7
ST72	***4***	7
ST70	***2***	3
ST6	***1***	2
ST7	***1***	2
ST36	***1***	2
ST37	***1***	2
ST8	***0***	0
ST10	***0***	0
ST15	***0***	0
ST16	***0***	0
ST17	***0***	0
ST18	***0***	0
ST23	***0***	0
ST27	***0***	0
ST28	***0***	0
ST30	***0***	0
ST31	***0***	0

Reference	Total score	%
ST34	***0***	0
ST39	***0***	0
ST40	***0***	0
ST41	***0***	0
ST44	***0***	0
ST48	***0***	0
ST49	***0***	0
ST52	***0***	0
ST53	***0***	0
ST56	***0***	0
ST57	***0***	0
ST58	***0***	0
ST59	***0***	0
ST60	***0***	0
ST61	***0***	0
ST62	***0***	0
ST63	***0***	0
ST66	***0***	0
ST68	***0***	0
ST71	***0***	0
ST74	***0***	0
ST75	*0*	0

STEC

Reference	Total score	%
STE102	**52**	87
STE348	**50**	83
STE347	**49**	82
STE218	**46**	77
STE149	**45**	75
STE328	**45**	75
STE68	**44**	73
STE94	**42**	70
STE18	**41**	68
STE70	**38**	63
STE208	**37**	62
STE15	**36**	60
STE221	**36**	60
STE224	**27**	45

Reference	Total score	%
STE344	**27**	45
STE349	**27**	45
STE71	**23**	38
STE113	**23**	38
STE346	**21**	35
STE226	**20**	33
STE65	**15**	25
STE9	***16***	27
STE69	***13***	22
STE6	***12***	20
STE29	***11***	18
STE52	***11***	18
STE345	***11***	18
STE31	***6***	10
STE212	***6***	10
STE343	***6***	10
STE57	***5***	8
STE60	***5***	8
STE184	***5***	8
STE204	***5***	8
STE251	***5***	8
STE252	***5***	8
STE253	***5***	8
STE10	***4***	7
STE50	***4***	7
STE350	***4***	7
STE13	***3***	5
STE26	***3***	5
STE30	***3***	5
STE14	***2***	3
STE5	***0***	0
STE7	***0***	0
STE8	***0***	0
STE12	***0***	0
STE16	***0***	0
STE17	***0***	0
STE19	***0***	0
STE20	***0***	0
STE21	***0***	0
STE22	***0***	0

Reference	Total score	%
STE23	***0***	0
STE24	***0***	0
STE25	***0***	0
STE28	***0***	0
STE32	***0***	0
STE33	***0***	0
STE34	***0***	0
STE36	***0***	0
STE37	***0***	0
STE38	***0***	0
STE39	***0***	0
STE40	***0***	0
STE41	***0***	0
STE42	***0***	0
STE46	***0***	0
STE47	***0***	0
STE48	***0***	0
STE49	***0***	0
STE51	***0***	0
STE53	***0***	0
STE54	***0***	0
STE58	***0***	0
STE59	***0***	0
STE61	***0***	0
STE66	***0***	0
STE73	***0***	0
STE111	***0***	0
STE270	***0***	0
STE329	***0***	0

APPENDIX 3

Supplementary Evidence Table for the biocontrol of *Listeria monocytogenes*

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific y/n	Limitations recorded	Reference details	
							ID	% Review score
RTE oven roast turkey breast	L. mono C391	Cocktail of 2 phages	n/a	Growth of phage treated samples during storage at 4°C following initial reduction.	n/a	Food matrix may hamper binding of target organism to phage	LI73	83
Soft cheese Minas Frescal, Coalho	L. mono raw milk cheese isolate and ScottA	Commercially produced single phage	lytic	ca 2 log drop in Lm observed after 7d on cheese stored at 10°C	Reported y on supplier website	Performance depnt on level of L. mono present	LI11	77
Melon, pear, apple, melon juice, pear juice, apple juice	L. mono cocktail CECT 4031, 940 and 4032	Commercially produced single phage	lytic	Growth of L. mono reported on fruit on storage at 10°C for 8d	Reported y on supplier website	Performance dependant on acidity and physical from of food matrix	LI21	77
Lettuce, apple, hard cheese, smoked salmon, frozen food	L. mono – Lm320 for lettuce, Lm68, 82 and 320 for other foods	Commercially produced cocktail of 6 phages	lytic	Further reductions in Lm reported on apples on storage but not cheese	Reported y on supplier website	Efficacy conc depnt Performance in apple pH depnt	LI119	77
Cooked turkey Roast beef	L. mono serotype 2x 1/2a, 1/2b, 4b	Commercially produced single phage	lytic	Phage levels remained relatively stable on surface of cooked turkey and roast beef for 28d at 4°C and 10°C	Reported y on supplier website	Inability of phage to reach target due to growth of L.m in protected niches. Performance improved with addition of antimicrobials	LI30	75

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific y/n	Limitations recorded	Reference details	
							ID	% Review score
Queso fresco cheese	L. mono 2 x 1/2a and 3x 4b	Commercially produced single phage	lytic	Phage levels remained relatively stable on cheese surface for 28d at 4°C	Reported y on supplier website	Inaccessibility of new targets in solid matrices	LI58	75
Fresh channel catfish fillets	L. mono EDG and ScottA	Commercially produced single phage	lytic	1.8-2.1 log reduction in infective phage after 10d at 4 and 10°C	Reported y on supplier website	Efficacy influenced by food matrix	LI84	75
Hot dog sausages, cooked sliced turkey meat, smoked salmon, mixed seafood, chocolate milk, mozzarella cheese, iceberg lettuce, cabbage	Individual cultures WSLC and Scott A	2 Single phages	lytic	0.6 log reduction on most foods except lettuce and cabbage were levels were lower by up to 1.2log	Reported to be broad host range	Activity can be L.m isolate specific	LI96	75
Brazilian fresh sausage	Single 1/2a isolate	Commercially produced single phage	lytic	Levels of phage maintained following storage for 10d at 4°C	Reported y on supplier website	L. mono may be shielded by the food matrix	LI74	72
Raw salmon fillet	Individual cultures of 1/2a and 4b	Commercially produced single phage	lytic	Phage still stable following storage for 10d at 4°C	Reported y on supplier website	Efficacy conc depndt. Study on lab isolates not those commonly found in processing Environment	LI90	63

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific y/n	Limitations recorded	Reference details	
							ID	% Review score
RTE chicken breast roll	Single isolate	Single isolate	lytic	No evidence of stability available	No specificity done	L mono control is temp and phage conc depndt	LI75	63
Beef bologna, beef frankfurters, roast beef, roast turkey, sliced ham, smoked turkey, turkey salami, turkey bologna, turkey frankfurters, turkey pastrami Lebanon bologna	Cocktail isolates 1/2a, 1/2b and 4b	cocktail of 6 phages	lytic	No stability data reported	Specificity claimed	None listed	LI106	63
Extended shelf life (ESL) milk	Single strain Lm	Single phage and single phage plus bacteriocin	n/a	Regrowth occurred after 8d at 4°C 50% phage resistant colonies recovered from FWLLm3 treated samples. Strains remained sensitive to FWLLm1	No specificity done	Potential for resistance to develop.	LI1	55

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific y/n	Limitations recorded	Reference details	
							ID	% Review score
Cooked ham	Cocktail 2 isolates	Commercially produced single phage	lytic	Growth occurred on storage vacuum packed at 10°C although treated samples had less growth than controls	Reported y on supplier website	Natural phage resistance reported	LI98	50
		Commercially produced single phage plus 10^3 antagonisitic L. sakeii	lytic	*L. sakeii* reduced the level of outgrowth post initial reduction in levels *L. mono* *****. and was minimal when samples were stored at 4°C				
Cold smoked salmon	Cocktail 5 isolates	Commercially produced single phage	lytic	Phage numbers maintained for 24 at 4°C with and w/o presence of either 20 000ppm LAE or 50 000ppm nisin	Reported y on supplier website	Phage activity is temperature and concn dependant Internalised L. mono may be out of reach of surface applied phage	LI14	40

Supplementary Evidence Table for the biocontrol of *Salmonella* spp.

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% Review score
Chicken skin (irradiated)	*S.* Enteritidis ATCC 13076 (x7 others used to analyse host range)	cocktail of x5 φSE phages	lytic	Not reported	Infective for7/ 7 serotypes tested. not infective for 6/7 non *Salmonella* strains	none recorded	SA88	90
Energy drink	*S.* Typhimurium LT2 (x18 others used to analyse host range)	Single P22	lytic	Greater reductions on storage for 48hrs for milk samples.	Infective against x12 *S.*Typhimurium, *S.* Derby & *S.* Enteritidis. Serotypes trialled that not affected = Newport, Muenchen & Muenster. no non-*Salmonella* culture results reported	Levels of *Salmonella* were affected by product type so approx 1 log reduction over the 48hours for the apple juice and energy drink with no significant difference in milk samples – may have affected log reductions observed for phage treated samples with less *Salmonella* present to reduce.	SA22	90
Apple Juice								
Whole milk								
Skimmed Milk								
Liquid whole egg								
Sliced chicken breast	*S.* Typhimurium LT2	Single P22				None reported		
Chicken Mince	*S.* Typhimurium LT2	Single P22				None reported		
Liquid whole egg	x1 *S.*Typhimurium isolate and x3 or x1 undefined *Salmonella* and	Single P22				Vicous nature of egg could reduce diffusion and homogenous distribution of phage		
Mung Bean	11 serovars of	Cocktail of 6	lytic	No stability data reported	Infective against	None reported	SA224	90

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% Review score
Alfalfa seeds	*Salmonella*	phages F01, P01, P102, P700, P800 & FL41			x11/11 *Salmonella* strains tested. No non-*Salmonella* culture results reported			
Pig Skin	*S.* Typhimurium U288	Cocktail of 4 phages; φSH17, φSH18, φSH19 & Felix 01,	lytic	Infective phage levels stable on pig skin over a 216 hour period tested	Infective against 15 /28 *Salmonella* strains tested. No non-*Salmonella* culture results reported	None reported	SA182	83
Soybean milk	*S.* Typhimurium ATCC 13311	Single ST1	lytic	Infective phage levels stable in product over a 24 hour period tested	Infective against 1 /2 *Salmonella* *strains tested. Infective against 1/16 non *Salmonella* strains (+ve for *Shigella dysentariae*)	Discrepency in data for the host range data.* More work on concentration of contaminated bacteria, food types, matrix and storage temperature required.	SA39	82

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% Review score
Chicken skin	*S.*Enteritidis SAL111-CF-KF10	Single wksl3	lytic	Some growth of Salmonella observed between days 1 and 2, then no significant growth. *Salmonella* cells from day 7 tested retained susceptibility to phage wksl3	Infective against 77/111*Salmonella* strains tested. No non-*Salmonella* culture results reported	Lack of contact between *Salmonella* and phage due to immobilisation on food could cause incomplete *Salmonella* reduction. Further work required on time points frequency and dosage on different food types.	SA97	82
Chicken skin	*S.* Montevideo	Single AV-08	lytic	No increase in *Salmonella* levels after day 1 (tested to day 6) observed. Reduced lytic effect on storage at 4ºC	Infective against *Salmonella* Montevideo and an *E. coli* O157:H7 strain. . No non-*Salmonella* culture results reported	Lytic affect may have occurred during 1 hour post treatment storage at 25ºC prior to storage at 4ºC	SA129	82

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% Review score
Hot dogs Cooked sliced turkey breast Mixed seafood Chocolate milk Egg yolk	*S.* Typhimurium DB 7155 stmR and DB 7155 camR	Single FO1-E2	lytic	Infectivity of phage was measured over 6 days; titres remained stable for all products except turkey which dropped to 50%. After 6 days phage insensitive colonies were found	No specificity reported	Lack of contact between *Salmonella* and phage due to immobilisation on food could cause incomplete *Salmonella* reduction	SA150	82
Dried pet food	*S.* Entitidis (ATCC 4931), *S.*Montevideo (ATCC 8387,) *S.* Senftenberg (ATCC 8400) & *S.* Typhimurium (ATCC 13311)	Cocktail SalmoLyse : SBA-1781, SPT-1, SSE-121, STML-198, STML-13-1 & SKML-39	lytic	No stability data reported	Infective against 882/940 *Salmonella* strains tested. No non-*Salmonella* culture results reported	Performance is affected by level of target organism and level of phage applied.	SA12	77
Tomatoes	*S.*Javiana 5913	Cocktail: F01, P01, P102, P700, P800	lytic	Phage levels decreased to below detection during storage of tomato plants between 0 and 14 days	No specificity reported	Performance is affected by storage temperature, further experiments revealed greater effect when stored at 30ºC in comparison to 15ºC Treatment may have been more successful if higher MOI had been used.	SA260	77

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% Review score
Broccoli Cantaloupe Strawberries	*S.* Saintpaul ATCC 9712 *S.* Paratyphi ATCC 51962 *S.*Typhimurium ATCC13311 *S.*Cholerae-suis ATCC 6958 *S.*Typhimurium ATCC 14028	Cocktail of 6 phage SalmoFresh	Lytic	No stability data reported	No specificity reported	none reported	SA73	63
Pig skin Chicken Eggs Lettuce	*S.* Typhimuriumm UA1872 (ATCC 14028) *S.* Enteritidis UA1894 (LK5)	Cocktail of 3 phages; UAB_φ20, UAB_φ78 UAB_87	Lytic	phage levels remained high throughout all treatments.	No specificity reported	Further evaluation needed under slaughterhouse conditions. Variation in egg shell surface could have led to variation in conc of *Salmonella* may affect phage performance.	SA84	63

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% Review score
Mixed salad leaves Beansprouts Cooked chicken breast Raw skinless chicken breast	*S.* Enteritidis PT4	Cocktail 3 phages: vB_Sens-Ent1, -Ent2 -Ent 3	Lytic	-Phage levels declined by 1 -1.5 log in mixed salad over 7 days. Also increase in levels of *Salmonella* observed from day 3. - Phage levels remained high in beansprouts, cooked and raw chicken throughout.	Specific to *Salmonella* serogroups A, B and D1	May have been improper mixing during treatment of mixed salad leaves. Inclusion of additional phages in any treatment would be required to extend coverage	SA281	57

Supplementary Evidence Table for the biocontrol of STEC

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% Review score
Green leaf lettuce (Modified atmosphere packaged)	1 strain E. coli O157:H7	Cocktail	lytic	-	-	Single host used, limited methods and data available	STE349	45
Spinach (Modified atmosphere packaged)	1 strain E. coli O157:H7	Cocktail	lytic	-	-	Single host used, limited methods and data available	STE349	45
Romaine lettuce (Modified atmosphere packaged)	1 strain E. coli O157:H7	Cocktail	lytic	-	-	Single host used, limited methods and data available	STE349	45
Chicken skin	1 strain E. coli O157:H7 ATCC4076	Single phage AV-08	Lytic	Infective phage levels are stable over the 5days stored	Infective against 1 strain of E. coli O157:H7, and 1 strain of Salmonella enterica sv. Montevideo no other data recorded	Single host (though tested E.coli mixed with Salmonella)	STE149	75
Cabbage	1 strain E. coli O157:H7 NCTC12079	Single phage ECP4	Lytic	-	-	Single host only	STE15	60
Vegetable juice	1 strain E. coli O157:H7 NCTC12079	Single phage ECP4	Lytic	-	-	Single host only	STE15	60

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% Review score
Fermented milk	x 1 E. coli strains: x 1 E. coli O157:H7 STEC464	Cocktail DT1 & DT6	Lytic	Infective phage levels reduced slightly throughout 24 hr by ≈ 1.6 log pfu/ml	see below 2 rows for information on host range of both phage	Single host only. The MOI of log 3 and 4 may not have been high enough	STE18	68
Fermented milk	x 3 E. coli strains: x 1 E. coli O157:H7 STEC464, x1 E. coli DH5α , x1 E. coli EPEC920	Single phage DT1	Lytic	Infective phage levels remained stable for first 8 hrs deceased by ≈ 1 -2 log pfu/ml between 8 and 24hr	DT1 Infective against x 1 E. coli O157:H7 STEC, x2 EPEC, x1 non-O157 STEC, x2 UDEC Not infective against 35 non-E.coli and nonpathogenic strains	Hosts tested in food singularly. The MOI of log 3 and 4 may not have been high enough	STE18	68
Fermented milk	x 2 E. coli strains:x 1 E. coli O157:H7 STEC464, x1 non 0157 STEC	Single phage DT6	Lytic	Infective phage levels remained stable for first 8 hrs deceased by ≈ 1 -2 log pfu/ml between 8 and 24hr	DT6 Infective against x 6 E. coli O157:H7 STEC, x4 EPEC, x3 non-O157 STEC, x3 UDEC Not infective against 35 non-E.coli and nonpathogenic strains	Hosts tested in food singularly. The MOI of log 3 and 4 may not have been high enough	STE18	68
Raw beef	1 strain of non toxigenic *E. coli* O157:H7 NZRM 3647	Single phage FAHEc1	Lytic	Not reported	-	Single host only	STE68	73

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% Review score
Sterilised milk	E. coli O157:H7	Cocktail of 2 phage: BECP2 and BECP6	Lytic	not reported	BECP2 and BECP6 infective against 27 & 23 *E. coli* O157:H7 strains respectively	Single host only	STE70	63
Strawberry	5 strains of E. coli O157:H7 229, 230, 231 (intralytix) ATCC43894 ATCC 35150	Cocktail of 3 phages – EcoShield BC	Lytic	None reported	No specificity reported	None reported	STE94	70
Cantaloupe							STE94	70
Broccoli							STE94	70
Sliced raw beef	E. coli O157:H7 ERL 033447 and non-pathogenic NZRM 3614	Single phage FAHEc1	Lytic	Y (at 37ºC)	Infective for E.coli O157:H7 28 out of 30 isolates and *E. coli* O162:H7of 29 other *E. coli* isolates were not infected. 13 non-*E.coli* cultures were all negative for lysis	Single host used for some experiments	STE102	87
Baby spinach	3 strains of E. coli O157:H7 (38, 39, 41, CEv2, AR1,42, ECA1, ECB7)	Cocktail x 8 strains BEC8	Lytic	Y	BEC8 phages capable of causing lysis of 94 – 98% of 123 strains	-	STE218	77
Baby romaine lettuce								

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% Review score
Spinach on harvester blades	5 strains of E. coli O157:H7	Cocktail of 6 strains	Lytic	no stability data reported	No specificity reported	None reported	STE221	60
Fresh cut cantaloupe Fresh cut iceberg lettuce	1 strain E. coli O157:H7	Cocktail of 3 strains ECML-4, ECML-117, ECML-134	Lytic	no stability data reported	no specificity reported	Single host only	STE328	75
Beef	5 strains of E. coli O157:H7	Cocktail of 4 strains, T5, T1, T4 & O1	Lytic	no stability data reported	no specificity reported	none recorded	STE344	45
Raw beef	1 strain E. coli O157:H7	Cocktail of 3 strains, EcoM-AG2, EcoM-AG3, EcoM-AG10	Lytic	-	infective for *E. coli* O157:H7, *E. coli* O26:H8	Single host only	STE208	62
							STE348 (same experiment as STE 208)	83
Raw beef	1 strain E. coli O157:H7	Cocktail of 3 strains, EcoM-AG2, EcoM-AG3, EcoM-AG10	Lytic	Tested over 9 days after 3 days *E.coli* numbers started to increase, colonies tested were still sensitive to the phage cocktail	infective for *E. coli* O157:H7, *E. coli* O26:H8	Single host only	STE347	82

Supplementary Evidence Table for the biocontrol of *Staphylococcus aureus*

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% Review score
Cheese	S. aureus	Cocktails (2x)	lytic	n	S. aureus, S. xylosus	Single host used	ST76	78
Cheese (Fresh)	S. aureus	Cocktail	lytic	n	-	Single host used	ST33	75
Cheese (Hard)	S. aureus	Cocktail	lytic	n	-	Single host used	ST33	75
Pasteurised whole milk	S. aureus	Cocktail	lytic	-	-	Data for combination of phages and HHP only. Single host.	ST35	73
Pasteurised whole milk	S. aureus	Cocktail	lytic	-	-	None identified	ST51	93
UHT whole fat milk	S. aureus	Single phages (x2)	temperate	-	-	Single host used	ST67	77
UHT whole fat milk	S. aureus	Cocktail	temperate	(Possibly)	-	Single host used	ST67	77
Pasteurised whole fat milk	S. aureus	Cocktail	temperate	-	-	Single host used	ST67	77
Semi-skimmed raw milk	S. aureus	Cocktail	temperate	-	-	Single host used	ST67	77
Whole fat raw milk	S. aureus	Cocktail	temperate	-	-	Single host used	ST67	77

Supplementary Evidence Table for the biocontrol of *Bacillus* spp.

Food product	Organism	Single phage/cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific y/n	Limitations recorded	Reference details	
							ID	% score
Cheonggukjang (fermented soyabean paste)	B. cereus	Single phage	lytic	-	B. cereus	Single host used	BA14	82
Cheonggukjang (fermented soyabean paste)	B. cereus	Single phage	lytic	-	B. cereus	Single host used, but did include competing flora	BA37	75
Mashed potato	B. cereus	Single phages (x2)	lytic	-	B. cereus, B. thuringiensis, B. mycoides, (B. licheniformis; possibly lysis from without only)	Single host used	BA35	82

Supplementary Evidence Table for the biocontrol of Enteric bacteria

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% score
UHT milk	ATCC25922	Cocktail 3 phage	n/a	None noted	n/a	Limitations on host range and raw milk not tested for native phages	ES83	73

Supplementary Evidence Table for the biocontrol of Lactic Acid Bacteria

Food product	Organism	Single phage /cocktail of phages	Mode of action lytic/lysogenic	Presence of residual activity y/n	Phage organism specific	Limitations recorded	Reference details	
							ID	% score
Beer	*L. brevis* 56	Single: SA-C12	Lytic	phage shown to be stable in beer over first 6 days. No increase in *L.brevis* detected over 4 day trial.	No specificity reported	none reported	LA45	65

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